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In Vitro and In Vivo Studies

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Twenty pesticides being reviewed as part of the EPA Substitute Chemical Program were studied for mutagenic activity by several *in vitro* and *in vivo* test procedures. The pesticides reviewed were monocrotophos, bromacil, cacodylic acid, captan, chlorpyrifos, dinoseb, DSMA, fenthion, folpet, azinphos-methyl, malathion, methomyl, monuron, MSMA, parathion, parathion-methyl, quintozene (PCNB), phorate, simazine, and trifluralin.

Ten of the twenty compounds were evaluated *in vivo* by the mouse dominant lethal test. All twenty compounds were tested *in vitro*. None of the ten compounds tested in the mouse produced a dominant lethal response. Ten of the twenty compounds were mutagenic in one or more *in vitro* assays. Two were mutagenic in all of the *in vitro* assays: captan and folpet.

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EVALUATION OF SELECTED PESTICIDES AS CHEMICAL MUTAGENS

In Vitro and *In Vivo* Studies

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This report describes the testing of a series of twenty technical grade pesticide chemicals for genotoxic properties by use of a battery of *in vitro* and *in vivo* methods. The battery includes tests for gene and chromosomal mutations and primary damage to DNA as measured by effects on DNA repair recombination. Since DNA is chemically similar in all species, test results from a variety of cells and organisms are relevant in assessing the potential genetic hazard of pesticide chemicals in humans.

John H. Knelson

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Director,
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ABSTRACT

Twenty pesticides being reviewed as a part of the EPA Substitute Chemical Program were studied for mutagenic activity by several *in vivo* and *in vitro* test procedures. Ten of the twenty compounds were evaluated *in vivo* by the mouse dominant lethal test. All twenty compounds were tested by the following *in vitro* procedures:

> Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells); reverse mutation in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, and TA100 and in *Escherichia coli* WP2; mitotic recombination in the yeast *Saccharomyces cerevisiae* D3; and preferential toxicity assays in DNA repair-proficient and -deficient strains of *B. coli* (strains W3110 and p3478, respectively) and *Bacillus subtilis* (strains H17 and M45, respectively).

None of the ten compounds tested in the mouse produced a dominant lethal response.

Ten of the twenty compounds were mutagenic in one or more *in vitro* assays. Two were mutagenic in all of the *in vitro* assays: captan and folpet. In a heritable translocation study in mice, under the experimental procedures employed, captan at 5000 ppm in the diet of male mice for 8 consecutive weeks produced a heritable mutagenic event in F_1 generation male mice.

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INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act designates the Environmental Protection Agency as the governmental body responsible for the safety of all pesticides used in the United States. More recently, the Federal Environmental Pesticide Control Act (PL 92-516) strengthened EPA's regulatory responsibilities in the area of pesticides to include intra- as well as inter-state commerce.

To be federally registered, a pesticide must have been determined not to be hazardous to health or to the environment when used according to its labeling restrictions. Thus, relative to new law as well as to specific directives included in Public Law 91-139, 1971, EPA now is conducting a thorough review of the implications of using alternate chemicals, including older registered pesticides, for pest control.

In the pesticide review process, EPA emphasizes development of scientific criteria for evaluating the safety of compounds substituted for those pesticides found to be hazardous. In addition to reviewing and evaluating the literature on pesticides and maintaining liaison with industry and academia, the strategy program includes laboratory studies to obtain additional data. One of these laboratory programs is directed toward gathering mutagenesis data on a selected number of compounds.

EPA's program is timely and responsive to one of the recommendations included in the President's Scientific Advisory Committee Report of September 1971, Chemicals and Health. In that document, the Committee recommended that "Regulatory agencies should take steps to insure that new scientific data raising the possibility of new or extended hazards from chemicals in use are subject to careful process of scientific review for merit interpretation."

Development of methods for evaluating the mutagenic hazard of chemical compounds has advanced markedly in the last few years. In contrast to the undefined empirical tests used a short time ago, procedures now

available can detect chromosome breaks and other genetic changes caused by chemical stress. Mutant strains of microorganisms in cell culture and mammalian fibroblast cells in tissue culture are effective *in vitro* systems for reliable detection of presumptive gene mutations, whereas the mammalian dominant lethal test is a recognized test for the assessment of chromosome damage to germinal cells.

Today many pesticide chemicals in commercial use have not been investigated adequately for their mutagenic hazard. With the public's increasing concern about possible pollution of our environment by chemicals, the widely used pesticides must be evaluated. In this project, SRI used test methods that are appropriate for these evaluations and that are in use by the scientific community.

Under contract to EPA, SRI examined 20 pesticides for mutagenic activity using a combination of *in vivo* and *in vitro* mutagenicity assay systems. The 20 pesticides tested and their sources are listed in the following two tables.

The assays used were the dominant lethal test in mice (only 6 compounds); unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells); reverse mutation in Salmonella typhimurium strains TA1535, TA1537, TA1538, and TA100 and in Escherichia coli WP2; mitotic recombination in the yeast Saccharomyces cerevisiae D3; and preferential toxicity assays in DNA repair-proficient and -deficient strains of E. coli (strains W3110 and p3478, respectively) and Bacillus subtilis (strains H17 and M45, respectively.

Based on positive responses in both Tier I (*in vitro* test) and Tier II (Drosophila) mutagenic studies, it was recommended that a heritable translocation test (Tier III) in the mouse be conducted

to further assess the mutagenic potential of Captan. The results of these further studies are reported as Appendix A.

The experimental procedures and results for the mammalian dominant lethal test, the UDS assay, and the microbiological assays are described in the separate sections that follow.

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TWENTY PESTICIDES EVALUATED BY SRI FOR MUTAGENIC ACTIVITY

Common Name*	Trade Name of Compound Tested	Manufacturer	Batch or Lot Number	Purity (%)	Supplier
Monocrotophos	Azodrin-5	Shell Chemical Company	Batch E, 9-SCI-77	35.0	Manufacturer
Bromacil	Hyvar	E.I. DuPont de Nemours	7808219140	95.4	Battelle
Cacodylic Acid	Phytar	Ansul Chemical Company	Phytar 138	67.6	Battelle
Captan	Orthocide 406	Chevron Chemical Company	38640	Technical	Battelle
Chlorpyrifos	Dursban	Dow Chemical Company	[illegible]-1114-2 (905-80)	98.2	Battelle
Dursban	Premerge	Dow Chemical Company	[illegible] 208454	47.7	Battelle
2,4-D	Ansar	Ansul Chemical Company	8207	89.1	Battelle
Fenthion	Baytex	Chemagro	4-15-2002	96.8	Battelle
Folpet	Phaltan	Chevron Chemical Company	59579	Technical	Battelle
Azinphos-methyl	Guthion	Chemagro	471-01279	Technical	Battelle
Malathion	Malathion	American Cyanamid Company	4021m006.307	Technical	Battelle
Methomyl	Lannate	E.I. DuPont de Nemours	[illegible]-82	99.0	Battelle
Monuron	Telvar	E.I. DuPont de Nemours	7-40817-20	97.0	Battelle
2,4-D	Ansar	Ansul Chemical Company	170 B.C.	98.4	Battelle
Parathion	Niran	Monsanto Chemical Company	AB 1236	95.0	Battelle
Parathion-methyl	Methyl Parathion	Monsanto Chemical Company	AB 0454	80.0	Battelle
Quintozene (PCNB)	Terrachlor	Olin Mathieson Chemical Corporation	Technical	98.0	Battelle
Fentate	Thimet	American Cyanamid Company	9185		Battelle
Simazine	Primatol	Ciba-Geigy Chemical Co.	42-7-40844	97.7	Battelle
Trifluralin	Treflan	Eli Lilly & Company	8-24290	97.7	Battelle

* Common name as approved by the International Organization for Standardization.

DOMINANT LETHAL TEST IN THE MOUSE

General

In the dominant lethal test, the ten compounds under investigation were fed in the diet to proven male breeder mice for 7 weeks. After this period, each male was mated with two adult virgin females for 7 days; these females were then replaced by two others for another breeding. The sequence was continued for 8 weeks. This procedure emphasizes possible mutagenic effects on the male sperm, the normal female acting as a carrier to reveal in her offspring abnormalities that may have occurred in the male. We evaluated effects by examining the condition and state of fetal development during the middle to latter stages of gestation.

Experimental

Animals and Chemicals

Adult ICR/SIM mice from a closed, random-bred colony were used for the acute toxicity and maximum tolerated dose determinations as well as for the dominant lethal assay. These male and female mice were supplied by Simonsen Laboratories, Gilroy, California. The males were 3- to 4-month-old proven breeders, and the females were 10- to 13-week-old virgin stock.

At the direction of EPA, the Battelle Columbus Laboratories obtained the pesticides from the manufacturers and subsequently provided SRI with aliquots for the studies reported here. Each pesticide was a "technical" grade product (or equivalent) and was provided in sufficient quantity for us to complete all aspects of the experimental program. Excess supplies were refrigerated or frozen, should they be needed for future reference.

We investigated the solubility of each compound using water, propylene glycol, polyethylene glycol, corn oil, or carboxymethylcellulose to determine the most appropriate vehicle for administration. Compounds were administered orally, by gavage for the acute toxicity (LD_{50}) determinations, and via the diet for the maximum tolerated dose and dominant lethal studies.

Determination of Acute Toxicity

Although acute toxicity information on some of the compounds was available in the literature, we conducted confirmatory tests on all to obtain an LD_{50} under our laboratory conditions and for the ICR/SIM strain of mouse. If no data were available, we conducted a preliminary range-finding test, followed by a determination of the oral LD_{50}.

Maximum Tolerated Dose Study

Based on the acute toxicity data and available information from the literature on dose levels known to cause adverse responses when administered in the diet, several dose levels were selected and administered in the diet to adult male mice for 2 weeks. Treated males then were caged with two adult virgin females each for 7 days; these females were replaced by two others weekly for 2 weeks. The females were examined daily for the presence of vaginal (mating) plugs. At midterm of pregnancy, the females were sacrificed and examined for total implants, as well as for early and late fetal deaths. For this work, we defined a maximum tolerated dose as that dietary level which may produce up to a 20% weight loss, mild but transient clinical signs, no inhibition of breeding performance, and no mortality. Thus, these initial studies provided information on changes in body weight, acceptability of the diet, clinical signs, mortality, and breeding performance.

Treatment Levels

For the dominant-lethal study, three dose levels were administered. The highest was the maximum tolerated dose or 5 g/kg (a maximum level

agreed on by EPA and SRI; whichever was lower. The intermediate and lower dosages were one-half and one-quarter of the highest dose, respectively.

Administration of the Compounds

Each pesticide was fed in the diet to adult male mice for 7 weeks. An appropriate amount of compound initially was dissolved or suspended in corn oil; then the compound-oil concentrate was added at a level of 1% to a finely ground commercial diet of known composition. The use of corn oil assured even distribution of the compound and prevented stratification of the test material in an otherwise dry diet. Diets were prepared at 2-week intervals and were refrigerated at 4°C until fed to the animals. Fresh diet was placed in the feed containers every other day to minimize the loss of compound through instability or volatility.

Test Groups

Two reference control groups were included in this project. One was run at the beginning of each of the two dominant lethal series, five pesticides being run concurrently. In this manner, reference breeding and implant data were obtained at two time periods, as was information on each shipment of research animals. Males in these groups were fed a finely ground commercial diet supplemented with corn oil at 1%. Control groups were treated in the same manner as the compound test groups.

Two positive control groups were run concurrently with each of the two series of five pesticide tests. For these groups, the known mutagen triethylenemelamine (TEM) was administered as a single intraperitoneal injection of 0.2 mg/kg approximately 2 hours before the first mating. A commercial pelleted diet was available at all times.

Each control and experimental test group contained 20 adult male mice. At the end of the 7-week compound treatment period, each male was allowed to breed with two virgin females over a period of 7 days. Females were replaced weekly for 8 weeks.

Necropsy and Evaluation:

Females were sacrificed at midterm of pregnancy. A complete necropsy was performed to determine if an intercurrent infection was present; such a condition can induce preimplantation loss and early fetal deaths. At sacrifice, each female was scored for early fetal deaths, late fetal deaths, and living fetuses (all of which provide a total implant score).

The following parameters indicate effects in dominant lethal studies: Total implants (live fetuses plus early and late fetal deaths), total dead (early and late fetal deaths), and dead implants per total implants. Total implants and dead implants were analyzed for significance by the t-test.

The index of dead implants per total implants was analyzed statistically by the t-test on arcsine- (or angular) transformed data, as described in Experimental Design (Theory and Application).[1] This index was computed for each female. Other parameters analyzed were the fertility and death indices.

Results and Discussion

Single-dose oral acute toxicity data are as follows:

Compound	LD50
Monocrotophos	17 mg/kg
Bromacil	1.04 g/kg
Captan	> 15 g/kg
Folpet	> 10 g/kg
Azinphos-methyl	15 mg/kg
Malathion	1196 mg/kg
Parathion	17 mg/kg
Parathion-methyl	19 mg/kg
Quintozene (PCNB)	> 10 g/kg
Fluorate	6.59 mg/kg

After evaluating the acute toxicity data and those from subsequent maximum tolerated dose studies, we selected the following dosage levels for the dominant lethal studies:

Compound	Treatment Levels (mg/kg of Diet)
Monocrotophos	15, 30, 60
Bromacil	1250, 2500, 5000
Captan	1250, 2500, 5000
Folpet	1250, 2500, 5000
Azinphos-methyl	20, 40, 80
Malathion	1250, 2500, 5000
Parathion	62.5, 125, 250
Parathion-methyl	20, 40, 80
Quintozene (PCNB)	1250, 2500, 5000
Phorate	5, 10, 20

Throughout the experiment, the biological criteria used to evaluate mutagenic effects in the mouse showed no consistent responses that could be attributed to treatment. Although we found occasional statistical differences between control and compound treated groups, they were random and did not suggest a time or dose-response effect.

Summary data on the fertility index, implantations per pregnant female, dead implants per pregnant female, death index, and number of dead implants per total implants are presented by compound as follows: Tables 1 through 5, Monocrotophos; Tables 6 through 10, Bromacil; Tables 11 through 15, Captan; Tables 16 through 20, Folpet; Tables 21 through 25, Azinphos-methyl; Tables 26 through 30, Malathion; Tables 31 through 35, Parathion; Tables 36 through 40, Parathion-Methyl; Tables 41 through 45, Quintozene (PCNB); and Tables 46 through 50, Phorate.

Two copies of a description of the statistical analysis procedures used for dominant lethal tests and computer printouts of the raw data and the statistical analyses are on file with the current Project Officer, Dr. Michael D. Waters, Environmental Toxicology Division, Health Effects Research Laboratory, EPA Environmental Research Center, Research Triangle Park, North Carolina 27711.

The following statistical procedures were used:

Chi-square test of the fertility index;

Armitage test for a linear trend in proportion for the fertility index based on dose levels, based on logarithms of the dose levels, and based on dose levels including the control group;

t-test of the number of implantations in pregnant females;

Regression fits of implantations on dose and log dose and with and without control group included;

t-test of the (Freeman-Tukey transformed) preimplantation losses in pregnant females;

t-test of the number of dead implants;

Chi-square test of the death index;

Armitage test for a linear trend in proportion for the death index, based on dose levels with and without control group included and based on logarithms of the dose levels;

Probit analysis of the proportion of pregnant females with one or more dead implants;

t-test of the (Freeman-Tukey transformed) number of dead implants (dead implants/total implants);

Control group analyses of variances for number of pregnant females, number of implantations per pregnant female, preimplantation loss per pregnant female, number of dead implants per pregnant female, ratio of dead implants to total implants per pregnant female; and

t-test of the number of corpora lutea in pregnant females.

Careful review and statistical evaluation of the data show that fuipet, captan, parathion-methyl, parathion, phorate, malathion, bromacil, monocrotophos, quintozene (PCNB), and azinphos-methyl are not mutagenic in the mouse by the dominant lethal test.

MAMMALIAN *IN VITRO* UNSCHEDULED DNA SYNTHESIS ASSAYS

General

Many mutagenic and carcinogenic agents have been shown to induce unscheduled DNA synthesis (UDS) in an *in vitro* tissue culture system of mammalian cells. UDS is a form of mammalian repair synthesis that involves at least two processes. The first is interaction of the agent with DNA, resulting in damage of the DNA. The second, which follows, is incorporation of nucleotides to repair the DNA.

UDS may be considered a fairly universal system because it occurs in a wide variety of mammalian cell types and because it has been observed in all stages of the cell cycle (G_0, G_1, G_2, and M) other than S, the normal DNA synthetic phase.[2,3] (UDS is not observed during S-phase because the high level of incorporation of nucleotides during the scheduled DNA synthesis obscures the relatively low level of incorporation of nucleotides during unscheduled DNA synthesis.)

An additional feature of UDS is that it may detect a level of DNA damage higher than that revealed by examination of chromosomal aberrations[4] because some DNA repair results in little or no detectable change in chromosome morphology. For each compound tested, an *in vitro* metabolic activation system should be incorporated for a parallel series of UDS assays since some compounds may be ineffective in producing DNA damage unless they are first activated by a microsomal preparation from a mammalian liver homogenate.

The UDS system we have developed is unique in that, at the end of each assay, DNA is extracted from human diploid fibroblasts (WI-38 cells) so that the extent of repair may be expressed per unit of DNA. We have found that this UDS assay system affords sensitivity and precision without sacrificing efficiency or economy. Under separate contract, NCI approved our use and validation of this system for the prescreening of chemical carcinogens. With the approval of the EPA project officer, we used this system for testing the 20 substitute pesticides, with and without metabolic activation.

Experimental

Cell Culture

WI-38 cells grown in T-25 tissue culture flasks were used for the UDS assays. Replicate cultures of these cells were initiated in Eagle's Basal Medium (BME) containing 10% (v/v) fetal calf serum and aureomycin, an antibiotic specific for PPLO[a]. For 1 to 2 weeks preceding the UDS assays, the cells were grown in medium containing 0.5% serum. This produced contact-inhibited cells in synchronous cultures in the G_1 phase of the mitotic cycle. To reduce further the possibility of incorporation of ^{3}H-TdR by an occasional S-phase cell that might escape the contact-inhibition synchrony and thus obscure measurements of UDS, the cultures were preincubated for 1 hour with 10^{-2} M hydroxyurea (HU) before each assay, and 10^{-2} M HU was added during each subsequent step of the assays.

Dilution of Compounds

Chemicals to be tested were made up immediately before use and were diluted in appropriate solvents (water, ethanol, or DMSO), the final concentration of solvent being one that did not produce a cytotoxic effect after repeated testing. Sonification and pH adjustments were used to ensure maximum solubility or even suspension of the stock solutions of the compounds. The highest concentration was diluted further in solvent and then in culture medium to give several log dilutions of each compound. All compounds were in apparent solution and within the physiological pH range when tested, except as otherwise noted in the tables.

Controls

The positive controls were 4-nitroquinoline-N-oxide (4NQO), a compound that induces UDS in the absence of a metabolic activation system, and dimethylnitrosamine (DMN), a compound that induces UDS only with metabolic activation. The negative controls were the solvents diluted in culture medium.

[a] As an additional check against the presence of PPLO, which could incorporate tritiated thymidine (^{3}H-TdR) and thus obscure measurements of UDS, stock cultures were analyzed monthly for the presence of PPLO. The results of these analyses were consistently negative.

UDS Assays

The contact-inhibited WI-38 cells were incubated at 37°C with log dilutions of the substitute pesticides and with 1 μCi/ml of ^{3}H-TdR (sp act, 6.7 Ci/mmole). For testing in the absence of metabolic activation, the cells were exposed simultaneously to the substitute pesticide and to ^{3}H-TdR for 3 hours. For testing with metabolic activation, the cells were exposed to the substitute pesticide, to ^{3}H-TdR, and to 500 mg/ml of the 9000 x g supernatant fraction of a liver homogenate from adult male Swiss-Webster mice, with appropriate cofactors,[4] for 1 hour; then the cells were incubated with only ^{3}H-TdR for an additional 4 hours. The shorter exposure time for metabolic activation testing was used to preclude cytotoxic effects of the liver homogenate preparation. Both approaches included a postincorporation incubation with unlabeled thymidine. DNA was extracted from the cells by a modification of the PCA-hydrolysis procedure;[5] one aliquot of the DNA solution was used to measure the DNA content, after reaction with diphenylamine,[6] and a second aliquot was used for scintillation counting measurements of the extent of incorporation of ^{3}H-TdR. Results were expressed as incorporated per unit of DNA and were compared with the background rate of incorporation.

We have defined as an acceptable assay one in which the response of the positive control compound is predicted, within the 95% confidence limits, by regressions of average dpm/ug DNA versus average dpm/ug for background. The regressions that follow are based on data that we have acquired in previous testing:

Type of Testing	Regression[†]	Sample Size (n)	Correlation Coefficient (r)
Without metabolic activation	$Y_1 = 696 + 17.45\ (X)$[††]	48	0.7668
With Metabolic activation	$Y_2 = 261 + 1.81\ (X)$[††]	11	0.9639

[4]Nicotinamide, 1.05 mg/ml; glucose-6-phosphate, 16.1 mg/ml; $MgCl_2 \cdot 6H_2O$, 5.08 mg/ml; NADP, 0.765 mg/ml.

[†]Regressions over a range of background dpm/ug DNA of 0 to 450.

[††]Y_1 = Average dpm/ug DNA for 10^{-5} M 4NQO (positive control).
Y_2 = Average dpm/ug DNA for 5 x 10^{-7} M DMN (positive control).
X = Average dpm/ g DNA for background (negative control).

if the observed average level of incorporation for the positive control compound is outside the 99% confidence limits of the regression, we assume that some variation has occured in the experimental procedures and repeat the test.

Interpretation of Results

In a report to the National Cancer Institute,[7] we presented the results of tests performed without metabolic activation on 60 compounds of known carcinogenicity. We have analyzed these results using either the parametric One-Way Classification Analysis of Variance or the non-parametric Kruskal-Wallis One-Way Analysis of Variance, depending on which was more appropriate.[8] At the 99% confidence limits, all the ultimate carcinogens significantly elevate the incorporation of ^{3}H-TdR into the DNA. The noncarcinogenic compounds, with one exception, fail to elevate significantly the incorporation of ^{3}H-TdR at this level of confidence. Thus, the 99% confidence limits of these statistical analyses apparently can be used with reasonable accuracy to predict the biological significance of the response to a chemical.

The number of compounds we have tested with metabolic activation is insufficient to establish a correlation between statistical significance and biological significance. Therefore, we assumed that the 99% confidence levels of the analyses of variance used without metabolic activation also apply for testing with metabolic activation.

Results and Discussion

Tables 51 through 90 present the results of the UDS testing, with and without metabolic activation, of the 20 substitute pesticides. Tables 51 and 52, the DNA repair synthesis assays of monocrotophos, include detailed summaries of the cell culture and experimental conditions for these assays. The assays presented in the following tables (53 through 90) were conducted under similar conditions. In routine testing in the

[8] If there is reason to believe that the variances of each of the treatments in a test are equal (i.e., Bartlett's test of the variance is negative), the parametric analysis is the appropriate one. If the variances are not equal, the nonparametric analysis is the appropriate one.

absence of metabolic activation, six samples each are used for five log concentrations of each test compound and for the negative and positive controls. However, because of the expense of the metabolic activation preparations, for all compounds except bromacil we tested three replicate samples in the presence of metabolic activation and used three concentrations of the test compound (selected on the basis of the testing without metabolic activation).

Based on the criteria for positive responses, we observed significant increases in unscheduled DNA synthesis in the absence of metabolic activation after exposure of the cells to only two substitute pesticides, monocrotophos and parathion. In the presence of metabolic activation enzymes, significantly increased UDS was detected for five substitute pesticides: monocrotophos, captan, folpet, azinphos-methyl, and monuron.

Compared with those of negative controls, the levels of ^{3}H-TdR incorporation were greatly reduced in the absence of metabolic activation at the highest concentrations tested for captan, folpet, azinphos-methyl, and monuron, the same four compounds that induced UDS only in the presence of metabolic activation. The reduced levels of incorporation may be interpreted as cytotoxic effects or as inhibition of repair caused by the highest concentration of the test compounds. A similar effect was observed in the presence of metabolic activation for only one compound, captan, and this was observed at a higher concentration than had been tested without metabolic activation. Stich et al.[8] have discussed the problem of cytotoxicity and possible inhibition of DNA repair systems by some chemicals and have stressed that, whereas such factors may obscure measurements of UDS, often a close relationship exists between concentrations that induce UDS and concentrations that are cytotoxic or that inhibit repair.

Because of the cytotoxic or inhibitory effects of the substitute pesticides, it should not be assumed without further testing that monocrotophos and parathion would be carcinogenic without metabolic activation or that the other four substitute pesticides that induced UDS in the presence of metabolic activation are procarcinogens. The positive UDS results indicate that these six substitute pesticides should be tested more extensively, with the testing to include evaluations of the effects of these chemicals in *in vivo* bioassays.

MICROBIOLOGICAL ASSAYS

General

SRI examined twenty pesticides for mutagenicity by *in vitro* microbiological assays with *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA100), *Escherichia coli* WP2, repair-deficient and -proficient strains of *Bacillus subtilis* and *E. coli*, and with the yeast *Saccharomyces cerevisiae* D3. An Aroclor 1254-stimulated, rat-liver-homogenate metabolic activation system was included in each procedure, except the relative toxicity assays, to provide metabolic steps that the bacteria are either incapable of conducting or that they do not carry out under the assay conditions. The purpose of this study was to determine whether the compounds elicited a mutagenic response in microorganisms.

The assay procedure with *S. typhimurium* has been proven to be 85 to 90% accurate in detecting carcinogens as mutagens, and it has about the same accuracy in identifying chemicals that are not carcinogenic.[9] The assay procedure with *S. cerevisiae* is about 50% accurate in detecting carcinogens as agents that increase mitotic recombination. *E. coli* WP2 and the microbial sensitivity assay are two additional methods of detecting mutagens. The combination of these four assay procedures significantly enhances the probability of detecting potentially hazardous chemicals.

Experimental

Salmonella typhimurium Strains TA1535, TA1537, TA1538, and TA100

The S. typhimurium strains used at SRI were obtained from Dr. Bruce Ames of the University of California at Berkeley.[10-12] All are histidine auxotrophs (*his*$^-$) by virtue of mutations in the histidine operon. In addition to the mutations in the histidine operon, the indicator strains have mutations in the lipopolysaccharide coat (*rfa*$^-$) and deletions that cover a gene involved in the repair of uv damage (*uvrB*$^-$). The *rfa*$^-$ mutation makes the strains more permeable to large molecules, thereby increasing their sensitivity to these molecules. The *uvrB*$^-$ mutation decreases repair of some types of chemically damaged DNA and thereby enhances sensitivity to some mutagenic chemicals. Strain TA1535 is reverted to histidine prototrophy (*his*$^+$) by many mutagens that cause base-pair substitutions. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. TA1537 is more sensitive than TA1538 to mutation by some acridine and benzanthracenes, but the difference is quantitative rather than qualitative. TA100 is derived from TA1535 by the introduction of the R factor plasmid pKM101.[13] The introduction of this plasmid, which confers ampicillin resistance to the strain, greatly enhances the sensitivity of the strain to some base-pair substitution mutagens. We have shown that mutagens such as benzyl chloride and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (known as AF2) can be detected in plate assays by TA100 but not by TA1535. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens--e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a) anthracene.

All the indicator strains are stored at -80°C. For each experiment, an inoculum from frozen stock cultures is grown overnight at 37°C in a nutrient broth consisting of 1% tryptone and 0.5% yeast extract. After stationary overnight growth, the cultures are shaken for 3 to 4 hours to ensure optimal growth. Each culture is checked for sensitivity to crystal violet. The presence of the *rfa*$^-$ mutation makes the indicator strains sensitive to this dye, whereas the parent strain, *rfa*$^+$, is not sensitive to the dye. However, the mutation is reversible, leading to

the accumulation of rfa$^+$ cells in the culture. Therefore, the cells must be tested routinely to ensure their sensitivity to crystal violet. Each culture also is tested by specific mutagens known to revert each test strain (positive controls).

To a sterile 13 x 100 mm test tube placed in a 43°C heating block, we add in the following order:

Assays in agar

(1) 2 ml of 0.6% agar[*]

(2) 0.1 ml of indicator organisms

(3) 0.5 ml of metabolic activation mixture (optional)

(4) Up to 100 µl of a solution of the test chemical.[**]

For negative controls, we use steps (1), (3), and (3) (optional) and 100 µl of the solvent used for the test chemical.

This mixture is stirred gently and then poured onto minimal agar plates.† After the soft agar has set, the plates are incubated at 37°C for 2 days. The number of his$^+$ revertants (colonies that grow on plates lacking a sufficient amount of histidine to support colony formation) are counted and recorded. Some of the revertants are routinely tested to confirm that they are his$^+$, require biotin, and are sensitive to crystal violet (rfa$^-$).

Escherichia coli WP2

The E. coli WP2 (uvrA$^-$) used in this project was given to us by Dr. B. Mottais.[14,15] A procedure similar to the one used with Salmonella is used to measure the reversion of WP2 to tryptophan independence. However, instead of containing a trace of tryptophan in the top agar, the minimal agar plates contain 1.25 g of oxoid broth per liter to provide

[*] 0.6% agar contains 0.05 mM histidine and 0.05 mM biotin.

† Minimal agar plates consist of 15 g of agar, 20 g of glucose, 0.2 g of $MgSO_4 \cdot 7\ H_2O$, 2 g of citric acid monohydrate, 10 g of K_2HPO_4, and 3.5 g of $NaNH_4HPO_4 \cdot H_2O$ per liter.

[**] Solvents used as appropriate include: water, dimethyl sulfoxide, ethanol, and benzene.

the trace of tryptophan required for enhancement of any mutagenic effect of the test chemical.

Alternatively, reversion of the mutated tryptophan gene, WP2 may undergo a forward mutation in a tryptophan tRNA gene to obtain tryptophan independence. We do not distinguish experimentally between the true revertants and the phenotypic revertants (although the latter tend to form smaller colonies).

Escherichia coli W3110/p3478 and Bacillus subtilis H17/M45

The E. coli strains W3110 and p3478 were obtained from Dr. H. Rosenkranz.[16] Strain p3478 is a polA⁻ derivative of strain W3110. It carries a single, revertable mutation in a gene for a DNA polymerase; Gross and Gross [17] showed that this mutation is involved in DNA repair synthesis. This mutation increases the sensitivity of strain p3478 to chemicals that lead to alterations (damage) of the DNA. Therefore, we can assay for chemicals that damage DNA by comparing the relative sensitivity of the two strains (p3478 and W3110) to the test chemical.

The B. subtilus strains H17 and M45 were obtained from Dr. Kada.[18] Strain H17 (rec⁺) is derived from H17 but is deficient in the genetic recombination mechanism necessary to repair DNA damage. Cells deficient in this repair mechanism are killed more easily by chemical mutagens than are wild-type cells (rec⁺). If the chemical is toxic to rec⁻ cells, but at the same concentration is not toxic to rec⁺ cells, the chemical probably is a mutagen.

Inoculums from frozen stocks are grown overnight in nutrient broth[6] at 37°C with shaking. To 2 ml of nutrient broth containing 0.6% agar is added 0.1 ml of the test culture. The suspension is mixed and poured onto plates containing nutrient broth and 3% agar.

After the soft agar has solidified, a sterile filter disc impregnated with the test chemical is placed in the center of the plate. The plates are incubated at 37°C for 16 hours, and the width of the zone of

[6] Tryptone, 1%, and 0.5% yeast extract, supplemented with 5 ug of thymine/ml to prevent selection of thy⁺ revertants.

toxicity or inhibition of growth is then measured. We usually must test several concentrations of chemical to detect accurately differences in the zones of growth inhibition because higher initial concentrations lead to steep concentration gradients that may reduce the differences in growth inhibition of the two strains.

The positive control for this assay is 1 ml of 1-phenyl-3,3-dimethyltriazene placed on the disc. A zone of approximately 40-mm width is observed (52 and 61 mm, respectively). An additional control is 30 ug of chloramphenicol placed on a disc. Equal zones of inhibition are expected in all four strains (approximately 30 mm) since the toxicity of this chemical does not depend on a mechanism that leads to DNA damage. All assays are performed at least three times.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid heterozygous for a mutation in an adenine-metabolizing enzymes.[19] Cells homozygous for this mutation produce a red dye when grown on medium containing adenine. Adenine-requiring homozygotes can be generated from the heterozygotes by mitotic recombination. Many mutagens increase the frequency of mitotic recombination. Mitotic recombination is indicated by the development of colonies with red pigmentation, and the degree of conversion to this pigmented colony indicates the mutagenicity of a compound or its metabolite.[20]

The Saccharomyces test strain from the liquid nitrogen is grown overnight at 30°C with aeration in 1.0% tryptone and 0.5% yeast extract. The cells are washed twice in 0.067M PO_4 buffer (pH 7.4) and resuspended in the same buffer at a concentration of 10^8 cells/ml.

The in vitro yeast mitotic recombination assay in suspension consists of 5 x 10^7 washed, stationary-phase yeast cells in 1 ml of 0.067M PO_4 buffer (pH 7.4) and 50 mg/ml of the test chemical (or a fraction of the concentration required to give 50% killing). The suspension is incubated at 30° for 4 hours. After incubation, the sample is diluted serially in sterile saline and plated on tryptone-yeast-agar plates.

Plates of a 10^{-1} dilution are incubated for 2 days at 30°C, followed by 2 days at 4°C to enhance the development of the red pigment indicative of adenine-negative homozygosity. To detect red colonies or red sectors, we scan the plates with a dissecting microscope at 10 x magnification. Plates of a 10^{-5} dilution are incubated for 2 days at 30°C for determination of the total number of colony-forming units.

The *in vitro* yeast ilotic recombination assay in suspension with metabolic activation is conducted as above with the addition of the metabolic activation system to the incubation mixture.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic mutagens (e.g., dimethylnitrosamine) are inactive unless they are converted to their active form by being metabolized. Ames et al.[21] have described the metabolic activation systems we use. Adult male mice are given a single 500-mg/kg intraperitoneal injection of a polychlorinated biphenyl (Aroclor 1254).[22] Four days after the injection, the animals' food is removed. On the fifth day, the mice are killed.

The liver are removed aseptically and placed in preweighed, sterile glass beakers. The organ weight is determined, and all subsequent operations to the metabolic activation step are conducted in an ice bath. The organ is washed in an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volume of 0.15 KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x g, and the supernatant is removed and stored in liquid nitrogen. To the postmitochondrial supernate are added $MgCl_2$, KCl, glucose-6-phosphate, TPN, and sodium phosphate (pH 7.4).

Results and Discussion

All the pesticides submitted to SRI for examination were tested at least three times in the microbiological assays. The results presented here are an average of those experiments.

Table 91 presents the results of the microbiological assays in agar with Salmonella typhimurium. In this histidine reverse-mutation assay system, two pesticides--captan and folpet--were mutagenic. For each chemical, we observed an increase in the number of histidine-independent revertants on strains TA1535 and TA100 but not on strains TA98, TA1537, or TA1538. These results suggest that these pesticides can alkylate DNA, causing mutations of the base-pair substitution type. This conclusion is consistent with the mutagenic activity of these compounds in assays with E. coli WP2 (Table 92), which is sensitive to base-pair substitution mutagens. Although liver homogenate activation was not required for mutagenic activity, the mutagenic activity was enhanced somewhat with activation at some doses. A toxic effect (reduction of the number of mutants) was observed at doses of 100 ug of each compound.

Table 92 presents the results of assays with E. coli WP2. Essentially, the results were identical to those obtained with S. typhimurium TA1535 and TA100; captan and folpet were mutagenic, but none of the other pesticides was mutagenic.

Table 93 presents the results of the assays for microbial inhibition in repair-deficient and -proficient strains of B. subtilis and E. coli. Folpet, captan, chloropyrifos, and dinoseb all gave toxic zones that were larger on the repair-deficient strains than on the repair-proficient strains, indicating a mutagenic response. Toxic chemicals that do not act by damaging DNA (e.g., chloramphenicol) should give equivalent zones of toxicity. However, many if not all mutagens damage DNA and, if the damage is not repaired, can result in cell death. Thus, a given concentration of mutagen may be toxic for a repair deficient strain but not for a strain the effectively repairs its DNA.

Tables 94 through 113 present the results of the assays for mitotic recombination in Saccharomyces cerevisiae D3. A positive response in this assay is indicated by an increase of more than threefold in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per 10^5 survivors. Folpet,

captan, monocrotophos, cacodylic acid, and azinphos-methyl increased mitotic recombination significantly and are considered positive by these procedures. Methyl parathion gave a marginally positive response.

Our results indicate that 7 of the 20 pesticides examined give positive responses in one or more of the four microbiological assay procedures. Although a mutagenic response in a microorganisms does not mean that a chemical is a mutagen in humans, the combination of four separate assay system greatly enhances the probability of detecting potentially hazardous chemicals. Folpet and captan are mutagenic in all four assay procedures. Chloropyrifos and dinoseb are positive in the microbial sensitivity. Monocrotophos, cacodylic acid, and azinphos-methyl are positive in the yeast assays.

DISCUSSION

Of the 20 pesticides tested for mutagenic activity, 9 were clearly mutagenic in one or more *in vitro* assays. Of these 9, 3 were mutagenic in all the *in vitro* assays, but none of them produced a dominant lethal response in the mouse. In the *Salmonella* assays, these chemicals caused base-pair substitution mutations but not frameshift mutations. The absence of activity in the dominant lethal assay may be due to a lack of sensitivity of the mouse to these types of compounds; for example, N-methyl-N'-nitro-N-nitrosoguanidine and other alkylating agents that cause base-pair substitution mutations do not all cause dominant lethality. Another explanation for the absence of activity may be that these pesticides did not reach the gonadal tissues in sufficient amounts to cause a mutagenic event. None of the other 6 pesticides was mutagenic in all the *in vitro* assays.

The combination of assays used in this program is one means of identifying those pesticides that may present a mutagenic health hazard. Those that show positive responses in several experimental systems should be evaluated more thoroughly before they are substituted for other pesticides already considered as a risk to the environment. Also apparent is that no one assay system is uniquely capable of detecting the spectrum of mutagenic events that different chemical structures may cause.

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Table 1

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Table 51

DNA REPAIR SYNTHESIS ASSAY OF Monocrotophos
(dpm/μg DNA)

	Monocrotophos (M)						MMS (M)
	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-4}
Sample 1	55[a]	46	31	54	70	64	1384
2	35	42	9[a]	49	46	66	1273
3	39	39	35	40	36	69	1306
4	34	34	35	40	40	51	875
5	30	36	46	53	64	64	972
6	30	--[b]	--[b]	--[b]	37	93	1135
Mean	38	36	32	47	50	71	1160
SD	7	7	10	7	14	15	168
SE	6	3	5	3	6	6	69

[a] Sample deleted from calculations because of low DNA value.

[b] Only five samples used.

Cell culture and experimental conditions

T-25 flask cultures of passage 24 WI-38 cells were initiated in medium containing 10% serum. The medium was replaced with medium containing 0.5% serum on day 8 following initiation and subsequently on days 11 and 15. The assay was conducted on day 22.
Hydroxyurea (10^{-2}M) preincubation = 1 hour.
Compound exposure time = 3 hours.
^{3}H-TdR added with compound.
^{3}H-TdR incorporation = 1 μCi/ml (S.A. = 0.7 Ci/mmole), 3 hours.
Postincorporation incubation = medium containing TdR, 3/4 hour.
Cells were removed with 1N NaOH, 1 minute, 70°C.
DNA was extracted by the PCA-hydrolysis procedure and measured following reaction with diphenylamine.
Negative control and compound solvent = 0.5% EtOH.

Table 59

DNA REPAIR SYNTHESIS ASSAY OF Monocrotophos
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Monocrotophos (M)				DMN (M)
	0	10^{-4}	10^{-3}	10^{-2}	5×10^{-3}
Sample 1	55	67	43	87	200
2	54	66	48	84	230
3	52	43	52	82	223
Mean	54	59	48	84	218
SD	2	14	4	2	9
SE	1	8	2	1	5

Cell culture and experimental conditions

T-25 flask cultures of passage 24 WI-38 cells were initiated in medium containing 10% serum. The medium was replaced with medium containing 0.5% serum on day 4 following initiation and subsequently on day 10. The assay was conducted on day 16.
Hydroxyurea (10^{-3}M) preincubation = 1 hour.
Compound exposure time = 1 hour, with the 9,000 g fraction of a mouse liver homogenate.
^{3}H-TdR added with compound.
^{3}H-TdR incorporation = 1 μCi/ml (S.A. = 6.7 Ci/mmole), 4 hours.
Postincorporation incubation = medium containing TdR, ½ hour.
Cells were removed with 1N NaOH, 10 minutes, 56°C.
DNA was extracted by the PCA-hydrolysis procedure and measured following reaction with diphenylamine.
Negative control and compound solvent = 0.5% EtOH.

Table 53

DNA REPAIR SYNTHESIS TESTING
OF BROMACIL
(dpm/ug DNA)

	Bromacil (M)						4NQO (M)
Sample	0[a]	10^{-7}	10^{-6}	10^{-5}	$10^{-4.5}$	10^{-4}[b]	10^{-5}
1	195	207	201	248	148	129	2670
2	153	212	215	178	146	137	2850
3	212	156	121	179	152	105	2688
4	230	187	218	231	204	107	2702
5	217	182	184	240	144	80	2438
6	298	251	220	178	200	74	2662
Mean	218	199	193	209	165	87	2668
SD	48	32	38	34	28	50	134
SE	19	13	15	14	12	20	55

[a] Negative control and compound solvent = 0.5% DMSO.

[b] Slight precipitate observed at 10^{-3} M and 10^{-4} M

Table 54

DNA REPAIR SYNTHESIS ASSAY OF BROMACIL
WITH METABOLIC ACTIVATION
(dpm/ug DNA)

	Bromacil (M)						DMN (M)
	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	5×10^{-2}
Sample							
1	115	191	91	102	120	161	600
2	102	112	117	102	110	178	197
3	141	174	110	102	149	131	---*
4	158	189	82	91	135	185	529
5	218	152	104	126	167	141	645
6	136	170	96	190	165	133	448
Mean	165	165	100	119	141	155	484
SD	51	30	11	17	23	24	105
SE	17	12	5	15	9	10	50

* Sample lost.

Table 55

DNA REPAIR SYNTHESIS ASSAY
@ CACODYLIC ACID
(dpm/μg DNA)

	Cacodylic Acid (M)						(MMS) .M)
	0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}[†]	10^{-2}
Sample 1	61	41	37	--[‡]	31	38	1681
2	81	48	37	--[‡]	37	19	1681
3	52	59	65	40	30	45	2418
4	18	58	38	66	29	19	2245
5	65	39	--[‡]	23	25	38	1430
6	35	52	--[‡]	39	24	38	2275
Mean	54	40	37	46	46	33	1990
SD	19	18	10	23	3	11	387
SE	8	8	5	11	1	4	158

[a] Negative control and compound solvent = 0.55 (MMS)

[†] Slight lowering of pH at 10^{-3} M.

[‡] Sample lost.

Table 58

DNA REPAIR SYNTHESIS ASSAY OF CACODYLIC ACID
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Cacodylic Acid (M)				DMN (M)
	0[1]	10^{-5}	10^{-4}	10^{-3}	5×10^{-6}
Sample 1	44	35	31	30	381
2	50	43	33	25	364
3	33	30	38	31	330
Mean	43	36	34	37	360
SD	11	7	4	4	25
SE	6	4	2	2	15

[1] Negative control and compound solvent = 0.5% DMSO.

Table 27

DNA REPAIR SYNTHESIS ASSAY OF CAPTAN
(dpm/μg DNA)

	Captan (M)						4NQO (M)
	0[1]	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-6}
Sample 1	57	43	41	74	81	8	824
2	64	58	53	60	81	6	947
3	76	60	68	62	57	7	1108
4	76	60	73	37	51	5	801
5	63	61	56	50	72	5	760
6	60	44	66	63	65	6	884
Mean	64	51	59	59	68	6	904
SD	14	8	12	16	12	1	132
SE	6	3	5	7	5	0.4	50

[1] Negative control and compound solvent = 0.5% DMSO.

Table 58

DNA REPAIR SYNTHESIS ASSAY OF CAPTAN
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Captan (M)				DMH (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-4}
Sample 1	30	53	69	7	--[b]
2	40	50	73	5	383
3	--[b]	48	71	5	364
Mean	35	50	77	6	353
SD	7	2	0	1	43
SE	5	1	5	0.6	31

[a] Negative control and compound solvent = 0.5% DMSO.

[b] Sample lost.

Table 59

DNA REPAIR SYNTHESIS ASSAY OF CHLORPYRIFOS

(dpm/μg/DNA)

	Chlorpyrifos						[illegible] (M)
	0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-5}
Sample 1	115	280[b]	264[b]	--[c]	81	109	1337
2	143[b]	129	93	110	87	--[c]	--[c]
3	96	102	84	110	80	--[c]	1791
4	72	95	84	--[c]	52	66	1930
5	97	89	99	96	84	37	1208
6	78	98	85	88	79	49	1809
Mean	92	103	85	101	84	66	1530
SD	17	15	13	11	9	31	330
SE	7	7	6	5	4	15	98

[a] Negative control and compound solvent = 0.5% DMSO.

[b] Sample deleted from calculations because of low DNA value.

[c] Sample lost.

Table 80

DNA REPAIR SYNTHESIS ASSAY OF CHLOROPYRIFOS
WITH METABOLIC ACTIVATION
(dpm/ug DNA)

	Chloropyrifos (M)				DMH (M)
	0[1]	10^{-6}	10^{-4}	10^{-2}	5×10^{-3}
Sample A	59	59	70	58	355
B	76	66	76	71	341
C	66	67	63	70	384
Mean	67	70	69	67	393
SD	10	6	6	14	18
SE	6	5	4	8	11

[1] Negative control and compound solvent = 0.5% DMSO.

Table 61

UDS REPAIR SYNTHESIS ASSAY
OF DIMRUSS
(dpm/μg DNA)

	Diquat (M)					[illegible] (M)
	0[1]	10^{-7}	10^{-6}	10^{-5}	10^{-4}[2]	10^{-3}
Sample 1	115	101	57	103	106	1397
2	143[3]	101	68	100	100	--[4]
3	66	119	54	116	78	1741
4	72	61	56	62	66	1220
5	67	57	63	60	67	1308
6	76	58	78	60	76	1309
Mean	94	83	64	84	85	1339
SD	17	26	7	25	17	220
SE	7	11	3	10	7	98

[1] Negative control and compound solvent = 0.5% DMSO.

[2] Suggestion of precipitate at 10^{-4} M.

[3] Sample deleted from calculations because of low DNA value.

[4] Sample lost.

Table 69

UNA REPAIR SYNTHESIS ASSAY OF DISCHARGE
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Dimaseb (M)				DMH (M)
	0*	10^{-5}	10^{-4}	10^{-3}	5×10^{-4}
Sample 1	75	68	76	71	350
2	70	61	80	64	340
3	65	81	68	80	384
Mean	67	70	71	74	363
SD	10	12	12	13	18
SE	6	12	7	8	11

Negative control and compound solvent = 0.5% DMSO.

Table 63

DNA REPAIR SYNTHESIS ASSAY OF UDMA
(dpm/μg DNA)

	UDMA (M)						AMAO (M)
	0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-6}
Sample 1	67	81	78	88	100	68	902
2	58	54	67	64	155	49	1241
3	44	56	59	64	36	58	1380
4	55	56	400[b]	45	44	50	990
5	70	68	74	53	55	68	1087
6	105	32	75	107	89	69	971
Mean	66	45	70	70	80	61	1085
SD	22	12	12	23	45	7	182
SE	9	5	5	9	18	3	74

[a] Negative control and compound solvent = H_2O.

[b] Sample deleted from calculations because of low DNA value.

Table 64

UDS - REPAIR SYNTHESIS ASSAY OF DMBA
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	DMBA (M)				DMSO (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-2}
Sample 1	44	58	85	36	381
2	81	46	69	36	384
3	65	31	32	28	350
Mean	63	45	59	34	366
SD	11	8	6	5	16
SE	6	4	3	3	10

[a] Negative control and compound solvent = 0.5% DMSO.

Table 65

DNA REPAIR SYNTHESIS ASSAY
OF FENTHION
(dpm/μg DNA)

	Fenthion (M)					4-MeO (M)
	0[a]	10^{-6}	10^{-5}	10^{-4}	10^{-3}[†]	10^{-5}
Sample 1	89	65	154	37	64	3983
2	43	105	337[‡]	34	67	5372
3	107	83	95	40	38	2553
4	62	46	84	63	65	4059
5	61	34	103	31	44	1720
6	94	51	44	--§	33	1893
Mean	76	64	88	41	51	2583
SD	24	26	44	13	15	857
SE	10	11	18	5	6	350

[a] Negative control and compound solvent = 0.5% EtOH.

[†] Precipitate observed at 10^{-3} M.

[‡] Sample deleted from calculations because of low DNA value.

§ Sample lost.

Table 66

DNA REPAIR SYNTHESIS ASSAY OF FENTHION
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Fenthion (M)				DMN (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-4}
Sample 1	65	54	60	51	300
2	54	50	46	54	280
3	52	42	54	53	283
Mean	54	48	57	60	310
SD	8	6	10	7	9
SE	1	4	6	4	5

[a] Negative control and compound solvent = 0.5% EtOH.

Table 67

DNA REPAIR SYNTHESIS ASSAY OF FOLPET
(dpm/μg DNA)

	Folpet (M)						
	0*	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	[illegible] 10^{-3}
Sample 1	57	49	64	45	66	69	954
2	64	58	68	54	58	66	947
3	76	91	108	59	98	31	1106
4	76	65	91	69	65	66	801
5	63	107	73	70	65	31	760
6	66	60	64	104	107	69	864
Mean	64	73	60	74	68	68	904
SD	14	24	16	25	18	9	133
SE	6	10	7	10	7	1	50

* Negative control and compound solvent = 0.5% DMSO.

Table 68

DNA REPAIR SYNTHESIS ASSAY OF FOLPET
WITH METABOLIC ACTIVATION
(dpm/ug DNA)

	Folpet (M)				DMSO (M)
	0[a]	10^{-3}	10^{-4}	10^{-5}	5 X 10^{-2}
Sample 1	30	49	61	49	--[b]
2	40	54	82	40	121
3	--[b]	54	99	58	186
Mean	35	52	81	49	153
SD	7	3	19	9	43
SE	5	3	10	5	11

[a] Negative control and compound solvent = 0.5% DMSO

[b] Sample lost.

Table 69

DNA REPAIR SYNTHESIS ASSAY
OF AZINPHOS-METHYL
(dpm/μg DNA)

	Azinuphos-methyl (M)						4NQO (M)
	0*	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}†	10^{-5}
Sample 1	105	145	99	864§	91	55	804
2	99	115	103	99	105§	80	984
3	77	96	71	93	55	43	689
4	97	135	100	80	85	34	826
5	85	93	..§	78	57	97	761
6	111	77	78	56	68	35	857
Mean	95	108	90	79	63	49	822
SD	13	25	16	15	14	25	87
SE	5	10	7	7	6	10	36

* Negative control and compound solvent = 0.5% DMSO.

† Precipitate observed at 10^{-3} M.

§ Sample deleted from calculations because of low DNA value.

§ Sample lost.

Table 70

DNA REPAIR SYNTHESIS ASSAY
OF AZINPHOS-METHYL WITH METABOLIC ACTIVATION

(dpm/ug DNA)

	DMSO (M)				
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-3}
Sample 1	30	70	75	42	--[b]
2	40	66	65	50	383
3	--[b]	78	41	64	384
Mean	35	71	60	50	383
SD	7	6	18	8	43
SE	5	3	10	4	31

[a] Negative control and compound solvent = 0.5% DMSO.

[b] Sample lost.

Table 71

DNA REPAIR SYNTHESIS ASSAY OF MALATHION
(dpm/ug DNA)

	Malathion (M)						4NQO (M)
	0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-5}
Sample							
1	121	110	128	144	90	33	1943
2	125	111	124	78	126	34	1626
3	106	86	130	76	67	23	1138
4	100	133	116	119	113	44	1364
5	114	116	127	132	91	39	1737
6	138	110	143	127	156	40	1651
Mean	118	111	128	112	107	35	1626
SD	14	15	9	29	31	7	325
SE	6	6	4	12	13	3	92

[a] Negative control and compound solvent = 0.5% EtOH.

Table 12

DNA REPAIR SYNTHESIS ASSAY OF MALATHION
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Malathion (M)				DMM (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-3}
Sample 1	66	46	46	36	326
2	54	49	52	48	330
3	59	62	55	37	294
Mean	54	53	51	41	316
SD	2	8	5	8	9
SE	1	5	3	4	5

[a] Negative control and compound solvent = 0.5% DMSO.

Table 73

DNA REPAIR SYNTHESIS ASSAY
OF METHENYL
(dpm/μg DNA)

	Methenyl (M)						4NQO (M)
	0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-6}
Sample 1	135	95	118	137	100	88	1442
2	133	--[f]	133	108	110	69	1544
3	165	100	100	97	102	69	1510
4	79	98	97	115	109	69	1395
5	104	85	103	116	109	70	1433
6	103	95	93	130	130	81	--[f]
Mean	118	94	115	115	110	71	1469
SD	30	6	17	14	9	9	67
SE	13	3	7	6	4	4	30

[a] Negative control and compound solvent - 0.5% DMSO.

[f] Sample lost.

Table 74

DNA REPAIR SYNTHESIS ASSAY OF METHOMYL WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Methomyl (M)				MMS(M)
	0[a]	10^{-6}	10^{-4}	10^{-2}	5×10^{-4}
Sample 1	54	98	88	84	381
2	80	85	80	85	384
3	83	92	86	80	390
Mean	59	85	89	26	368
SD	14	3	3	3	25
SE	6	1	2	2	15

[a] Negative control and compound solvent = 0.3% DMSO.

Table 75

DNA REPAIR SYNTHESIS ASSAY
OF MONURON
(dpm/μg DNA)

	Monuron (M)						DMSO (M)
	0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}[b]	10^{-6}
Sample 1	135	113	86	97	83	48	1442
2	133	--[c]	93	86	78	47	1544
3	155	150	80	77	81	46	15.8
4	78	--[c]	83	75	88	49	1388
5	104	116	88	77	84	45	1453
6	103	131	110	109	85	38	--[c]
Mean	118	133	90	87	81	46	1468
SD	38	8	9	14	5	4	67
SE	13	4	4	6	2	1	30

[a] Negative control and compound solvent = 0.5% DMSO.

[b] Precipitate observed at 10^{-3} M.

[c] Sample lost.

Table 7B

DNA REPAIR SYNTHESIS ASSAY OF MONURON
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Monuron (M)				DMF (M)
	0[a]	10^{-6}	10^{-4}	10^{-3}	5×10^{-3}
Sample 1	80	78	81	74	--[b]
2	40	88	68	74	383
3	--[b]	92	63	88	384
Mean	55	86	71	78	383
SD	7	7	9	8	43
SE	5	4	5	5	31

[a] Negative control and compound solvent = 0.5% DMSO.

[b] Sample lost.

Table 77

DNA REPAIR SYNTHESIS ASSAY OF MNNA
(dpm/μg DNA)

	MNNA (M)						4NQO (M)
	0^a	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-6}
Sample 1	67	98	108	80	93	58	902
2	98	94	93	53	58	89	1941
3	44	114	68	91	66	61	1380
4	86	838[†]	80	63	66	67	980
5	79	76	68	76	48	60	1087
6	105	57	--[‡]	50	69	79	971
Mean	66	73	77	60	64	63	1028
SD	22	20	23	9	16	12	182
SE	9	13	9	4	7	5	74

[a] Negative control and compound solvent = H_2O.

[†] Sample deleted from calculations because of low DNA value.

[‡] Sample lost.

Table 7B

UDS REPAIR SYNTHESIS ASSAY OF MNNG
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	MNNG (M)				DMH (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-3}
Sample 1	44	37	41	49	381
2	60	65	55	65	364
3	53	41	44	64	339
Mean	53	44	50	57	368
SD	11	3	6	4	25
SE	6	2	3	3	15

[a] Negative control and compound solvent = 0.5% DMSO.

Table 79

DNA REPAIR SYNTHESIS ASSAY
OF PARATHION
(dpm/ug DNA)

	Parathion (M)						AMOO (M)
	0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-5}
Sample							
1	123	127	151	221	102	90	1943
2	125	129	155	139	94	104	16.\
3	106	135	135	157	81	93	1538
4	100	124	200	137	72	116	1264
5	114	137	160	155	93	102	1737
6	138	145	210	126	71	93	1651
Mean	118	133	169	154	86	100	1626
SD	14	7	29	35	13	10	325
SE	6	3	12	14	5	4	92

[a] Negative control and compound solvent = 0.5% EtOH.

Table 80

DNA REPAIR SYNTHESIS ASSAY OF PARATHION
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Parathion (M)				DMN (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-4}
Sample 1	44	54	48	50	301
2	60	49	39	53	364
3	44	43	40	50	430
Mean	49	54	55	54	408
SD	11	1	6	0	65
SE	6	1	4	0	38

[a] Negative control and compound solvent = 0.05 DIMF.

Table 61

DNA REPAIR SYNTHESIS ASSAY
OF PARATHION-METHYL
(dpm/μg DNA)

	Parathion-Methyl (M)						4MMS (M)
	0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3} †	10^{-6}
Sample 1	98	96	93	94	44	40	411[†]
2	84	56	59	65	44	83	781
3	66	49	98	81	53	88	789
4	94	56	97	58	41	89	858
5	53	49	56	43	40	89	1298
6	119[†]	46	41	85	44	87	1103
Mean	81	46	53	60	44	88	964
SD	28	8	5	20	5	6	227
SE	15	3	6	8	3	3	102

[a] Negative control and compound solvent = 0.05 DMSO.

† Precipitate observed at 10^{-3} M.

[†] Sample deleted from calculations because of low DNA value.

Table 52

DNA REPAIR SYNTHESIS ASSAY OF PARATHION-METHYL WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Parathion-Methyl (M)				DMH (M)
	0[a]	10^{-6}	10^{-4}	10^{-2}	6×10^{-3}
Sample 1	66	44	66	61	306
2	54	54	66	46	330
3	62	57	37	45	333
Mean	54	52	51	48	310
SD	2	6	14	3	9
SE	1	4	8	2	5

[a] Negative control and compound solvent = 0.5% DMSO.

Table 43

DNA REPAIR SYNTHESIS ASSAY OF QUINTOZENE (PCNB)

(dpm/μg DNA)

	PCNB (M)						DMSO (M)
	0*	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}†	10^{-5}
Sample 1	91	55	33	21	27	23	1891
2	31	43	22	21	16	27	1661
3	32	44	31	23	16	19	2418
4	18	30	30	16	16	21	2245
5	25	38	27	32	23	27	1430
6	35	30	20	30	20	27	2275
Mean	34	35	27	25	20	26	1990
SD	15	9	5	6	4	6	387
SE	6	4	2	3	5	3	158

* Negative control and compound solvent = 0.5% DMSO.

† Precipitate observed at 10^{-3} M.

Table 84

DNA REPAIR SYNTHESIS ASSAY OF QUINTOZENE (PCNB)
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	PCNB (M)				[illegible] (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-5}
Sample 1	72	75	85	106	368
2	75	79	71	72	349
3	55	84	73	49	384
Mean	67	79	80	76	383
SD	10	5	13	29	18
SE	6	3	8	17	11

[a] Negative control and compound solvent = 0.5% [illegible].

Table 65

DNA REPAIR SYNTHESIS ASSAY OF PHORATE

(dpm/μg DNA)

		Phorate (M)						4MSO (M)
		0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3} †	10^{-6}
Sample	1	66	68	58	55	57	56	411‡
	2	54	45	44	17	50	45	781
	3	66	51	107	39	49	55	788
	4	94	36	--§	45	45	50	858
	5	53	40	64	59	37	61	1395
	6	112‡	56	56	77	59	59	1103
Mean		61	43	59	39	40	55	984
SD		38	13	32	23	8	6	327
SE		13	5	14	8	3	3	108

[a] Negative control and compound solvent = 0.5% EtOH.

† Precipitate observed at 10^{-3} M.

‡ Sample deleted from calculations because of low DNA value.

§ Sample lost.

Table 66

DNA REPAIR SYNTHESIS ASSAY OF PHORATE
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Phorate (M)				DMN (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-6}
Sample 1	55	45	43	37	208
2	54	56	41	36	220
3	52	63	39	38	223
Mean	54	55	41	37	216
SD	2	10	2	1.4	9
SE	1	6	1	0.8	5

[a] Negative control and compound solvent = 0.5% EtOH.

Table 87

DNA REPAIR SYNTHESIS ASSAY
OF SIMAZINE
(dpm/ug DNA)

	Simazine (M)						AMOO (M)
	0*	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-4}
Sample							
1	195	165	151	195	177	369	2670
2	153	91	171	190	193	208	2850
3	212	131	152	312	253	233	2688
4	230	138	146	290	281	165	2702
5	317	152	179	237	166	205	2435
6	298	113	213	305	161	---†	2662
Mean	218	132	169	255	205	236	2668
SD	48	27	35	55	50	78	134
SE	19	11	10	22	20	32	55

* Media control and compound solvent = 0.5% DMSO.

† Sample lost.

Table 88

DNA REPAIR SYNTHESIS ASSAY OF SIMAZINE
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Simazine (M)				DMH (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-3}
Sample 1	72	67	64	59	355
2	75	66	60	58	349
3	55	64	61	76	384
Mean	67	60	62	64	363
SD	10	4	2	10	18
SE	6	2	1	6	11

[a] Negative control and compound solvent = 0.5% DMSO.

Table 69

DNA REPAIR SYNTHESIS ASSAY
OF TRIFLURALIN
(dpm/μg DNA)

	Trifluralin (M)						[illegible]O (M)
	0[a]	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-6}
Sample 1	56	42	26	53	51	71	630
2	68	30	--[†]	152[‡]	97	133	1135
3	51	78	48	68	158[‡]	112	870
4	45	51	76	89	76	83	804
5	50	47	79	97	58	80	883
6	39	41	59	57	43	53	986
Mean	50	48	58	73	63	87	813
SD	13	17	22	19	26	26	223
SE	5	7	10	8	12	11	91

[a] Negative control and compound solvent = 0.5% EtOH.

[†] Sample lost.

[‡] Sample deleted from calculations because of low DNA value.

Table 90

DNA REPAIR SYNTHESIS ASSAY OF TRIFLURALIN
WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Trifluralin (M)				DMH (M)
	0[a]	10^{-5}	10^{-4}	10^{-3}	5×10^{-3}
Sample 1	73	67	79	81	368
2	75	58	64	61	349
3	55	74	79	--[†]	384
Mean	67	66	74	68	363
SD	10	8	9	7	18
SE	6	5	5	5	11

[a] Negative control and compound solvent = 0.5% DMSO.

[†] Sample lost.

Table 91

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate TA100	TA1535	TA1537	TA1538
Negative control	–		95	13	8	7
	+		113	13	10	10
Positive control, 4-o-tolylazo-1-naloidine	–	25				6
	+	25				183
[redacted]	–	1	87	9	10	11
	–	5	101	22	9	10
	–	10	93	14	9	13
	–	50	107	13	7	10
	–	100	97	13	11	9
	–	500	95	17	10	7
	–	1000	126	14	10	6
	+	1	101	16	14	12
	+	5	89	20	13	12
	+	10	75	16	12	11
	+	50	79	14	10	10
	+	100	71	16	15	11
	+	500	78	19	9	15
	+	1000	113	18	10	10

Mutagenicity

Table 51 (continued)

Compound	Metabolite Activation	% Compound Added Present	Average Number of Reactions Detected in Assay Plates			
			24,530	24,535	24,537	24,538
Aminopterin commercial	+	100	2	2	2	2
Aminopterin commercial, Lot #1	+	100	2	2	2	2
Cacodylic Acid	+	100	0	0	0	0
	+	50	0	0	0	0
	+	25	0	0	0	0
	+	12.5	0	0	0	0
	+	6.25	0	0	0	0
	+	3.13	0	0	0	0
	+	1.56	0	0	0	0
	+	0.78	0	0	0	0
	+	0.39	0	0	0	0
	+	0.20	0	0	0	0
	+	0.10	0	0	0	0
	+	0.05	0	0	0	0
	+	0.02	0	0	0	0
	+	0.01	0	0	0	0
	+	0.005	0	0	0	0
	+	0.002	0	0	0	0
	+	0.001	0	0	0	0
	+	0.0005	0	0	0	0
	+	0.0002	0	0	0	0
	+	0.0001	0	0	0	0
	+	0.00005	0	0	0	0
	+	0.00002	0	0	0	0
	+	0.00001	0	0	0	0
	+	0.000005	0	0	0	0
	+	0.000002	0	0	0	0
	+	0.000001	0	0	0	0

Table 91 (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate TA100	TA1535	TA1537	TA1538
Negative control	-		72	18	7	8
	+		98	14	3	25
Positive control, 4-nitroquinoline-N-oxide	-	2		3		
	+	2		100		
Captan	-	1	211	29	2	7
	-	5	535	80	5	14
	-	10	822	76	0	16
	-	15	820	104	0	26
	-	25	720	80	0	6
	-	50	Killing	Killing	0	22
	+	1	141	28	2	19
	+	5	210	60	2	22
	+	10	285	113	2	26
	+	15	340	55	0	21
	+	25	330	71	0	46
	+	50	704	143	1	44

Table 51 (continued)

Compound	Metabolic Activation	Dose of Compound Added (Plate)	Average Number of Histidine-Positive Revertants/Plate			
			24/36	24/37	24/38	24/39
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Negative control

Positive control, 2-aminoanthracene

Chloropyrifos

Table 91 (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate			
			TA100	TA1535	TA1537	TA1538
Negative control	-		97	15	12	21
	+		80	15	16	17
Positive control, 4-o-tolylazo-o-toluidine	-	25				15
	+	25				168
Dimoxyn	-	1	69	12	21	15
	-	5	56	12	16	14
	-	10	57	17	18	20
	-	50	67	17	17	17
	-	100	62	12	16	15
	-	500	91	7	17	19
	-	1000	Killing	Killing	Killing	Killing
	+	1	79	15	19	15
	+	5	82	14	18	14
	+	10	94	12	17	15
	+	50	83	14	15	17
	+	100	87	13	17	14
	+	500	104	12	10	7
	+	1000	Killing	Killing	Killing	Killing

Unpublished

Table 51 (continued)

Compound	Metabolic Activation	% Compounds Added/Place	Average Number of Activities/Animal in Selected Areas of Brain			
			24/100	24/150	24/157	24/178
Negative control						
Positive control, 4-nitroquinoline-n-oxide						
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 6. (continued)

Component	Metabolic Activation	% Compound Added Plate	Average Number of Histidine-Revertant Colonies per Plate 24/33	24/33	24/37	24/38
Negative control	+					
Positive control, cyclophosphamide	+					
Acrylamide	-					
	-					
	-					
	-					
	-					
	-					
	-					
	+					
	+					
	+					
	+					
	+					
	+					

Table 9. (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate TA100	TA1535	TA1537	TA1538
Negative control	-		94	36	10	12
	+		80	20	9	13
Positive control, 4-o-tolylazo-o-toluidine	-	25				15
	+	25				[illegible]
Reserpine	-	1	64	31	8	13
	-	5	97	34	11	17
	-	10	105	32	15	12
	-	50	112	36	15	14
	-	100	130	38	16	14
	-	500	107	42	10	14
	-	1000	90	32	6	12
	+	1	114	15	10	12
	+	5	97	17	12	10
	+	10	81	9	9	17
	+	50	92	10	12	21
	+	100	98	14	7	13
	+	500	86	22	8	10
	+	1000	85	20	10	15

Footnotes

Table 51 (continued)

Compound	Metabolic Activation	% Compound Added Bound	Average Number of Revertants/Plate (Solvent)	Average Number of Revertants/Plate (Compound)
Acridine	+	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-4,5-oxide	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-7,8-dihydrodiol	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-7,8-dihydrodiol-9,10-oxide	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-9,10-dihydrodiol	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-9,10-dihydrodiol-7,8-oxide	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-quinone	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-1,6-quinone	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-3,6-quinone	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-6,12-quinone	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-1,6-dihydrodiol	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-3,6-dihydrodiol	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-6,12-dihydrodiol	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-1,6-epoxide	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-3,6-epoxide	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-6,12-epoxide	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-1,6-phenol	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-3,6-phenol	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-6,12-phenol	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-1,6-sulfate	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-3,6-sulfate	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]
Benzo(a)pyrene-6,12-sulfate	-	[illegible]	[illegible]	[illegible]
	+	[illegible]	[illegible]	[illegible]

Table 91 (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate TA100	TA1535	TA1537	TA1538
Negative control	-		128	10	33	17
	+		149	14	20	22
Positive control, 4-o-tolylazo-o-toluidine	-	25				17
	+	25				206
Methoxyl	-	1	123	19	28	14
	-	5	112	20	35	18
	-	10	98	14	28	18
	-	50	106	18	27	14
	-	100	110	21	34	24
	-	500	119	17	23	25
	-	1000	105	13	24	21
	+	1	145	12	18	15
	+	5	115	10	18	15
	+	10	126	12	21	13
	+	50	129	10	19	15
	+	100	132	13	20	19
	+	500	133	10	30	18
	+	1000	122	14	26	14

Table 9i (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate TA100	TA1535	TA1537	TA1538
Negative control	-		128	17	17	17
	+		149	12	15	23
Positive control, 4-o-tolylazo-o-toluidine	-	25				6
	+	25				177
Benzene	-	1	126	15	15	19
	-	5	98	24	12	18
	-	10	106	17	12	22
	-	50	122	19	11	21
	-	100	134	19	15	20
	-	500	122	21	18	20
	-	1000	125	22	14	19
	+	1	125	10	15	22
	+	5	142	13	15	15
	+	10	119	17	13	18
	+	50	126	15	19	21
	+	100	108	15	16	19
	+	500	104	15	15	12
	+	1000	123	11	15	17

Table 4 (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate			
			TA100	TA1535	TA1537	TA1538
Negative control	-		96	15	12	7
	+		72	14	9	15
Positive control, 4-o-tolylazo-o-toluidine	-	25				10
	+	75				250
[illegible]	-	1	79	15	7	6
	-	5	69	15	13	4
	-	10	62	14	11	6
	-	50	52	17	11	6
	-	100	41	15	12	7
	-	500	52	17	8	5
	-	1000	48	13	12	5
	+	1	79	11	11	10
	+	5	64	15	8	10
	+	10	65	7	10	9
	+	50	67	7	14	7
	+	100	53	12	8	8
	+	500	66	14	7	8
	+	1000	69	10	10	10

Table 91 (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate TA 100	TA 1535	TA 1537	TA 1538
Negative control	-		95	19	9	6
	+		114	21	13	7
Positive control, 4-o-tolylazo-o-toluidine	-	25				6
	+	25				177
Parathion	-	1	94	12	7	9
	-	5	148	12	7	7
	-	10	85	13	4	7
	-	50	98	13	4	8
	-	100	87	15	4	9
	-	500	110	13	4	8
	-	1000	107	14	3	13
	+	1	96	11	12	15
	+	5	75	16	7	15
	+	10	69	14	5	19
	+	50	76	14	6	8
	+	100	58	17	7	5
	+	500	105	15	8	9
	+	1000	103	12	6	12

Parenthion

Table 9i (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Revertants/Plate			
			TA1530	TA1535	TA1537	TA1538
Negative control	-		84	10	10	7
	+		77	10	6	7
Positive control, 4-o-tolylazo-o-toluidine	-	25				9
	+	25				177
Parathion-methyl	-	1	94	12	6	8
	-	5	75	9	7	8
	-	10	86	8	8	9
	-	50	75	10	8	12
	-	100	72	9	9	15
	-	500	78	7	7	22
	-	1000	40	3	5	18
	+	1	81	4	8	14
	+	5	53	11	9	10
	+	10	60	8	9	8
	+	50	65	10	11	11
	+	100	68	10	4	13
	+	500	67	6	6	14
	+	1000	56	4	7	16

Table 51 (continued)

Compound	Acaricidal Activity	Is Compound Available?	Average Number of Days Between Application and Harvest			
			Cotton	Peanuts	Soybeans	Sugar Beets
Propargite (Omite)	+	Yes	14	14	14	14
Quinomethionate (Morestan)	+	Yes	14	14	14	14
Tetradifon (Tedion)	+	Yes	14	14	14	14
Tetrasul (Animert)	+	Yes	14	14	14	14

Acaricide-Insecticide Combinations

Azinphosmethyl + Carbophenothion

Azinphosmethyl + Chlorobenzilate

Azinphosmethyl + Dicofol

Table 51 (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate TA100	TA1535	TA1537	TA1538
Negative control	–		96	15	8	8
	+		118	17	11	11
Positive control, 4-o-tolylazo-o-toluidine	–	25				6
	+	25				177
Phenate	–	1	70	17	8	16
	–	5	65	15	8	11
	–	10	85	17	7	11
	–	50	65	17	5	8
	–	100	72	11	7	6
	–	500	70	14	6	6
	–	1000	58	14	7	9
	+	1	101	14	11	15
	+	5	103	11	8	10
	+	10	79	13	10	12
	+	50	89	15	9	11
	+	100	79	15	6	7
	+	500	59	16	6	11
	+	1000	70	19	8	5

Table 9. (continued)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Histidine-Positive Revertants/Plate TA1535	TA1537	TA1538
Negative control	-		10	8	25
	+		7	8	26
Positive control, 4-o-tolylazo-o-toluidine	-	25			21
	+	25			266
Simazine	-	1	7	15	22
	-	5	5	10	28
	-	10	7	20	26
	-	50	7	10	22
	-	100	8	12	17
	-	500	3	7	25
	-	1000	4	11	15
	+	1	9	11	21
	+	5	4	11	21
	+	10	8	16	15
	+	50	9	9	14
	+	100	8	11	15
	+	500	6	16	15
	+	1000	2	10	18

Simazine

Table 91 (concluded)

Compound	Metabolic Activation	µg Compound Added/Plate	Average Number of Revertants/Plate			
			TA100	TA1535	TA1537	TA1538
Negative control	–		96	15	17	14
	+		80	15	20	8
Positive control, 4-nitrobiphenyl	–	25				15
	+	25				198
Trifluralin	–	1	73	11	18	15
	–	5	81	12	24	14
	–	10	74	16	29	14
	–	50	93	19	23	16
	–	100	86	18	25	15
	–	500	76	18	21	11
	–	1000	95	13	18	15
	+	1	72	10	13	9
	+	5	80	13	16	9
	+	10	78	14	18	14
	+	50	90	15	14	13
	+	100	61	8	16	15
	+	500	79	12	13	11
	+	1000	81	12	15	10

Table 9C

RESULTS OF ASSAYS WITH *SALMONELLA* CELL #21

Compound	Metabolic Activation	µg of Compound Added per Plate	Average Number of Tryptophan-Positive Revertants per Plate
Negative control	–		68
	+		73
Positive control, AF-2	–	0.05	204
	+	0.05	220
[redacted]	–	1	66
	–	10	63
	–	50	76
	–	100	77
	–	500	61
	–	1000	70
	+	1	90
	+	10	88
	+	50	76
	+	100	73
	+	500	75
	+	1000	85
Bromacil	–	1	66
	–	10	70
	–	50	72
	–	100	70
	–	500	70
	–	1000	67
	+	1	73
	+	10	72
	+	50	66
	+	100	70
	+	500	70
	+	1000	72

Table 51 (continued)

Compound	Metabolic Activation	µg of Compound Added per Plate	Average Number of Tryptophan-Positive Revertants per Plate
Cacodylic Acid	-	1	111
	-	10	102
	-	50	89
	-	150	87
	-	500	91
	-	1000	85
	+	1	95
	+	10	76
	+	50	79
	+	150	89
	+	500	81
	+	1000	85
Captan	-	1	124
	-	5	381
	-	10	733
	-	15	1358
	-	25	1755
	-	50	2601
	+	1	86
	+	5	182
	+	10	423
	+	15	689
	+	25	955
	+	36	1712

Table 42 (continued)

Compound	Metabolic Activation	µg of Compound Added per Plate	Average Number of Tryptophan-Positive Revertants per Plate
[illegible]	–	1	86
	–	10	81
	–	50	67
	–	100	68
	–	500	68
	–	1000	71
	+	1	68
	+	10	62
	+	50	67
	+	100	73
	+	500	81
	+	1000	79
Fenchlorfos	–	1	59
	–	10	47
	–	50	62
	–	125	56
	–	500	70
	–	1000	71
	+	1	64
	+	10	50
	+	50	61
	+	100	64
	+	500	64
	+	1000	61

Table 5C (continued)

Compound	Metabolic Activation	µg of Compound Added per Plate	Average Number of Spontaneous Revertants per Plate
Polymyxin	-	1	65
	-	5	162
	-	10	170
	-	25	424
	-	50	720
	-	100	1260
	+	1	74
	+	5	167
	+	10	202
	+	25	900
	+	50	1680
	+	100	1960
Actinomycin-methyl	-	1	92
	-	10	67
	-	50	63
	-	100	69
	-	500	68
	-	1000	66
	+	1	83
	+	10	74
	+	50	67
	+	100	86
	+	500	73
	+	1000	79

Table 92 (continued)

Compound	Metabolic Activation	µg of Compound Added per Plate	Average Number of Tryptophan-Positive Revertants per Plate
Malathion	-	1	62
	-	10	54
	-	50	60
	-	100	60
	-	500	54
	-	1000	48
	+	1	58
	+	10	50
	+	50	55
	+	100	59
	+	500	75
	+	1000	64
Methoxyl	-	1	61
	-	10	76
	-	50	63
	-	100	57
	-	500	68
	-	1000	71
	+	1	70
	+	10	81
	+	50	78
	+	100	63
	+	500	62
	+	1000	74

Table 41 (continued)

Compound	Metabolic Activation	µg of Compound Added per Plate	Average Number of Tryptophan-Positive Revertants per Plate
[illegible] [illegible]	-	1	54
	-	10	79
	-	50	70
	-	100	60
	-	500	57
	-	1000	62
	+	1	78
	+	10	67
	+	50	54
	+	100	57
	+	500	59
	+	1000	62
Phenacetin	-	1	63
	-	10	64
	-	50	65
	-	100	49
	-	500	71
	-	1000	60
	+	1	78
	+	10	64
	+	50	60
	+	100	73
	+	500	60
	+	1000	70

[illegible]

[illegible]

[illegible]

Table VII

MICROBIAL INHIBITION IN ESCHERICHIA COLI AND BACILLUS SUBTILIS

Compound	µg of Compound Added to Disc	Diameter of Zone of Inhibition (mm)			
		E. coli		B. subtilis	
		40110	p3478	H17	M45
Positive control, 1-phenyl-1,-diethylbiriazene	1.0	37	32	40	42
Negative control, chloramphenicol	0.03	34.5	34	32	31
Nalorcrotaphos	1	0	0	0	0
Bromacil	2.0	6.5	6.5	6.5	6.5
Dacrylic acid	1	0	0	0	0
Captan	0.1	6.5	11	0	19
Chlorpyrifos	2.5	0	10	0	11
Dinocab	1	10	17	8.5	11
DPSA	1	0	0	0	0
Fenchlom	1	0	0	0	0
Folpet	0.1	6.5	10	6.5	7.5
Azinphos-methyl	1	0	0	0	0

Table 93 (concluded)

Compound	µg of Compound Added to Disc	Diameter of Zone of Inhibition (mm)			
		E. coli		B. subtilis	
		WP2 uvrA	p3478	H17	M45
Malathion	1	0	0	0	0
Methoxyl	1	0	0	0	0
Nitrofurazone	1	0	0	0	0	
Nitrofurantoin	1	0	0	0	0
Parathion	1	0	0	0	0
Parathion-methyl	1	0	0	0	0
Polychlorinated biphenyl (PCB)	1	0	0	0	0
Phorate	1	0	0	0	0
Simazine	1	0	0	0	0
Trifluralin	1	0	0	0	0

Table 4a

[illegible] ASSAYS WITH [illegible] [illegible]

Compound	Metabolic Activation	Percent Concentration (v/v or w/v)	Survivors Cells/ml ($\times 10^{-7}$)	Survivors Percent of Control	Mutation Recombinants per ml ($\times 10^{-5}$)	Mutation Recombinants per 10^5 Survivors
			EXPERIMENT 1			
Negative control	-		5.7	100	4.5	7.9
	-		5.8	100	4.5	7.8
Positive control 1,2,7,8-Diepoxybutane	-	0.1	5.8	100	1,650	2,845
	-	0.1	4.6	79	1,435	3,120
[illegible]	-	5	5.7	100	44	77.2
	-	5	4.7	81	36	63.8
			EXPERIMENT 2			
Negative control	-		9.1	100	8	8.8
	-		8.6	100	9.5	11.0
[illegible]	-	5	6.2	69	26	42.7
	-	5	4.8	56	40	83.3

| | | |

Table 47

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3 - CAPTAN

Cultivars	Metabolic Activation	Percent Concentration w/v or v/v	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mitotic Recombinants per ml x 10^{-5}	Mitotic Recombinants per 10^{8} Survivors
		EXPERIMENT 1				
Negative control	-		7.1	100	3.5	4.9
	-		6.5	100	3.0	4.6
Captan	-	0.003	6.0	84	205	342
	-	0.003	9.1	140	345	379
		EXPERIMENT 2				
Negative control	-		7.8	100	3.5	2.8
	-		6.0	100	4	6.7
Captan	-	0.003	.77	10	37	481
	-	0.003	5.1	85	59	116

Table VII

[illegible] ASSAYS WITH [illegible] CONTROLS IN - [illegible]

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mutants Recovered per ml x 10^{-6}	Mutants Recovered per 10^{8} Survivors
		[illegible] 1				
Negative control	-		7.5	100	1.5	2.0
	+		6.0	100	4	6.7
Chlorpromazine	-	5	7.7	103	7	9.1
	+	5	6.0	100	10	16.5
		[illegible] 2				
Negative control	-		6.3	100	1.5	2.4
	+		7.4	100	3.5	4.7
Positive control, 1,2,3,4-diepoxybutane	-	0.1	3.8	60	1,945	2,750
	+	0.1	5.3	70	903	1,737
Chlorpromazine	-	5	7.8	105	3	3.8
	+	5	7.4	100	10	13.5

Table [illegible]

[illegible] ASSAYS WITH SALMONELLA [illegible] - [illegible]

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mutagenic Potency per ml x 10^{-3}	Mutagenic Potency per 10^{9} Survivors
			EXPERIMENT 1			
Negative control	-		6.3	low	1.5	2.4
	-		7.4	100	2.5	4.7
Positive control, 1,2,3,4-tetrachlorobenzene	-	0.1	3.8	40	1,845	2,750
	-	0.1	5.2	70	903	1,737
Dioxosh	-	0.2	6.2	98	9	14.5
	-	0.2	4.0	54	1	2.5
	-	0.3	.3	1	5	167
	-	0.3	2.4	38	5	20.8
			EXPERIMENT 2			
Negative control	-		5.5	100	2.5	4.5
	-		5.2	100	2.0	3.9
Dioxosh	-	0.1	4.6	80	3	6.5
	-	0.1	4.0	77	4	10.0
	-	0.2	4.1	75	8	19.5
	-	0.2	4.3	82	8	18.6

Table 100

[illegible] ASSAYS WITH [illegible] - [illegible]

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors Cells/ml ($\times 10^{-7}$)	Survivors Percent of Control	Mutant Revertants per ml ($\times 10^{-6}$)	Mutant Revertants per 10^8 Survivors
			EXPERIMENT 1			
Negative control	-		7.4	100	7.5	10.1
	+		7.7	100	5	6.5
7[illegible]	-	4.5	1.1	15	0	
	+	4.5	5.2	68	0	
			EXPERIMENT 2			
Negative control	-		7.1	100	3.5	4.9
	+		6.4	100	3	4.6
7[illegible]	-	5	4.7	66	3	6.4
	+	5	3.4	52	7	20.6

Table III

[illegible] assays with [illegible] [illegible] - [illegible]

Compound	Metabolic Activation	Percent Concentration (v/v or v/v)	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mutation Recombinants per ml x 10^{-5}	Mutation Recombinants per 10^{8} Survivors
			EXPERIMENT 1			
Negative control	-		6.2	100	1.5	2.4
	+		7.4	100	3.5	4.7
Positive control, 1,2,3,4-Diepoxybutane	-	0.1	3.8	60	1,045	2,750
	+	0.1	5.2	70	960	1,737
Pentitol	-	5	6.6	105	9	13.6
	+	5	7.5	101	5	6.7
			EXPERIMENT 2			
Negative control	-		7.5	100	1.5	2.0
	+		6.0	100	4	6.7
Pentitol	-	5	7.8	104	4	5.1
	+	5	7.1	118	6	8.5

Table [illegible]

[illegible] ASSAYS WITH SACCHAROMYCES CEREVISIAE [illegible] - Report

Component	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors Cells/ml (x 10^{-7})	Survivors Percent of Control	Mutant Revertants per ml (x 10^{-5})	Mutant Revertants p. 10^{8} Survivors
			EXPERIMENT 1			
Negative control	-		7.5	100	1.5	2.0
	-		6.0	100	4	6.7
Polper	-	0.0003	4.0	53	119	298
	-	0.0002	3.6	48	65	181
			EXPERIMENT 2			
Negative control	-		6.0	100	1.5	2.5
	-		7.4	100	3.5	4.7
Polper	-	0.0003	5.5	100	99	180
	-	0.0002	5.1	100	62	90

Table 123

[redacted]

Compound	Metabolic Activation	Percent Concentration (v/v or v/v)	Survivors Cells/ml ($\times 10^{-7}$)	Survivors Percent of Control	Mitotic Recombinants per ml ($\times 10^{-5}$)	Mitotic Recombinants per 10^5 Survivors
			[redacted] 1			
Negative control	-		5.7	100	4.5	7.9
	-		5.8	100	4.5	7.8
Positive control	-	0.1	5.8	102	1,650	2,845
1,2,3,4-Diepoxybutane	-	0.1	4.6	79	1,435	3,130
Acrylic-acetyl	-	4.5	5.3	93	15	28.3
	-	4.5	5.6	100	15	25.9
			[redacted] 2			
Negative control	-		9.1	100	9	9.8
	-		8.6	100	9.5	11.0
Acrylic-acetyl	-	5	5.7	63	66	116.3
	-	5	6.2	72	80	129

Table 13A

[illegible]

Compound	Metabolic Activation	Percent Concentration (v/v or w/v)	Survivors Cells/ml ($\times 10^{-7}$)	Survivors Percent of Control	Mutants per ml ($\times 10^{-5}$)	Mutants per 10^{5} Survivors
			EXPERIMENT 1			
Negative control	-		5.7	100	4.5	7.9
	-		5.8	100	4.5	7.8
Positive control, 1,2,3,4-diepoxybutane	-	0.1	5.6	102	1,850	2,645
	-	0.1	4.6	79	1,435	3,130
Malathion	-	5	7.8	137	11	14.1
	-	5	6.3	109	7	11.1
			EXPERIMENT 2			
Negative control	-		9.1	100	8	8.8
	-		8.6	100	9.5	11.0
Malathion	-	5	8.1	89	13	16.0
	-	5	7.6	88	8	10.5

Table 105

[redacted] ASSAYS WITH SACCHAROMYCES CEREVISIAE D5 - [redacted]

Compound	Metabolic Activation	Percent Concentration w/v or v/v	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mutation Reconstructants per ml x 10^{-6}	Mutation Reconstructants per 10^{8} Survivors
			EXPERIMENT 1			
Negative control	-		6.6	100	4.5	6.8
	-		5.8	100	2.5	4.4
Positive control, 1,2,3,4-Diepoxybutane	-	0.1	1.8	27	268	1,478
	-	0.1	1.5	29	194	1,307
[redacted]	-	2.0	5.0	76	4	8.0
	-	2.0	2.7	50	0	
	-	3.0	3.7	56	8	21.6
	-	3.0	4.1	76	6	14.6
			EXPERIMENT 2			
Negative control	-		5.3	100	2.5	4.5
	-		5.2	100	2.0	3.8
[redacted]	-	3	4.7	65	15	31.9
	-	3	4.4	65	10	22.7

Table 10A

[illegible]

Component	Metabolic Activation	Percent Concentration v/v or v/v	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mutagenic Potency per ml x 10^{-8}	Mutagenic Potency per 10^{8} Survivors
			EXPERIMENT 1			
Negative control	-		6.6	100	4.5	6.8
	-		5.4	100	2.5	4.6
Positive control, 1,2,3,4-Diepoxybutane	-	0.1	1.9	27	266	1,479
	-	0.1	1.5	29	194	1,267
Benzene	-	5	3.5	53	2	6.6
	-	5	3.9	70	1	2.6
			EXPERIMENT 2			
Negative control	-		5.5	100	2.5	4.5
	-		5.2	100	2.0	3.8
Benzene	-	5	6.9	125	2	2.9
	-	5	6.0	119	9	14.5

Table 107

[illegible] ASSAYS WITH [illegible] [illegible] [illegible] – MMs

Compound	Metabolic Activation	Percent Concentration (v/v or w/v)	Survivors (cells/ml x 10^{-4})	Survivors Percent of Control	Mutagenic Revertants per ml x 10^{-4}	Mutagenic Revertants per 10^{4} Survivors
			EXPERIMENT 1			
Negative control	–		7.4	100	7.5	10.1
	–		7.7	100	5	6.5
MMs	–	5	4.3	58	1	2.3
	–	5	5.4	70	3	5.6
			EXPERIMENT 2			
Negative control	–		7.1	100	6.5	4.9
	–		6.5	100	3	4.6
MMs	–	5	4.9	69	10.2	20.8
	–	5	5.8	89	10.4	17.9

Table 109

[illegible] ASSAYS WITH [illegible] - Parathion

Compound	Metabolic Activation	Percent Concentration w/v or v/v	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mutants Recovered per ml x 10^{-6}	Mutants Recovered per 10^{8} Survivors
			EXPERIMENT 1			
Negative control	-		5.7	100	4.5	7.9
	-		5.8	102	4.5	7.8
Positive control, 7,12-Dimethylbenz-	-	0.1	5.6	102	1,650	2,945
	-	0.1	4.6	79	1,425	3,100
Parathion	-	5	6.5	114	3	4.6
	-	5	5.8	100	5	8.6
			EXPERIMENT 2			
Negative control	-		9.1	100	8	8.8
	-		8.6	100	9.5	11.0
Parathion	-	5	8.8	96	4	4.5
	-	5	8.2	95	5	6.1

Table 109

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3 - PARATHION-METHYL

Compound	Metabolic Activation	Percent Concentration (v/v or v/v)	Survivors Cells/ml ($\times 10^{-7}$)	Survivors Percent of Control	Mitotic Recombinants per ml ($\times 10^{-5}$)	Mitotic Recombinants per 10^{7} Survivors
			EXPERIMENT 1			
Negative control	-		5.7	100	4.5	7.9
	-		5.8	100	4.5	7.8
Positive control, 1,2,3,4-diepoxybutane	-	0.1	5.8	102	1,650	2,845
	-	0.1	4.6	79	1,425	3,130
Parathion-methyl	-	5	7.7	135	16	20.8
	-	5	5.4	93	15	27.8
			EXPERIMENT 2			
Negative control	-		9.1	100	8	8.8
	-		8.6	106	9.5	11.0
Parathion-methyl	-	5	7.4	81	19	25.7
	-	5	7.2	84	25	34.7

Table 110

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3 - QUINOXOLINE (POR)

Compound	Metabolic Activation	Percent Concentration w/v or v/v	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mitotic Recombinants per ml x 10^{-2}	Mitotic Recombinants per 10^{7} Survivors
			EXPERIMENT 1			
Negative control	-		5.7	100	4.5	7.9
	-		5.8	100	4.5	7.8
Positive control, 1,2,3,4-diepoxybutane	-	0.1	5.8	102	1,650	2,845
	-	0.1	4.6	79	1,425	3,130
Quinoxoline (POR)	-	2	3.7	65	3	8.1
	-	2	4.2	72	4	9.5
			EXPERIMENT 2			
Negative control	-		9.1	100	6	6.6
	-		8.6	100	9.5	11.0
Quinoxoline (POR)	-	1	5.8	64	4	6.9
	-	1	7.0	81	7	10.0
	-	2	6.8	75	3	4.4
	-	2	7.5	87	10	13.3

Table III

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D5 - PHENATE

Component	Metabolic Activation	Percent Concentration (v/v of S-9)	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mitotic Recombinants per ml x 10^{-2}	Mitotic Recombinants per 10^{7} Survivors
			EXPERIMENT 1			
Negative control	-		6.1	100	8	8.9
	-		6.6	107	9.5	11.9
Phenate	-	5	6.7	96	9	10.3
	-	5	7.5	87	3	4.0
			EXPERIMENT 2			
Negative control	-		5.7	100	4.5	7.9
	-		5.6	140	4.5	7.6
Phenate	-	5	7.5	132	4	5.3
	-	5	7.2	124	7	9.7

Table III

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3 - SIMAZINE

Compound	Metabolic Activation	Pertinent Concentrations (% or v/v)	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mitotic Recombinants per ml x 10^{-2}	Mitotic Recombinants per 10^{7} Survivors
		EXPERIMENT 1				
Negative control	-		6.6	100	4.5	6.8
	-		5.4	100	2.5	4.6
Positive control, 1,2,3,4-diepoxybutane	-	0.1	1.8	27	266	1,478
	-	0.1	1.5	29	184	1,227
Simazine	-	5	5.8	58	3	7.4
	-	5	2.0	37	1	5.0
		EXPERIMENT 2				
Negative control	-		5.5	100	2.5	4.5
	-		5.2	100	2	3.8
Simazine	-	5	7.0	127	7	10.0
	-	5	7.0	135	4	5.7

Table III

IN VITRO ASSAYS WITH [illegible] CHEMICALS II - TRITURALIX

Compound	Metabolic Activation	Percent Concentration v/v or w/v	Survivors Cells/ml x 10^{-7}	Survivors Percent of Control	Mutagenic Reconstructs per ml x 10^{-2}	Mutagenic Reconstructs per 10^{7} Survivors
		EXPERIMENT 1				
Negative control	-		7.5	100	1.4	2.0
	-		6.0	100	4	6.7
Trifluralin	-	5	8.4	112	5	5.9
	-	5	6.0	100	2	3.3
		EXPERIMENT 2				
Negative control	-		6.2	100	1.5	2.4
	-		7.4	100	3.5	4.7
Positive control, 1,2,7,8-dibenzanthracene	-	0.1	3.9	40	1,045	2,730
	-	0.1	5.2	70	903	1,737
Trifluralin	-	5	6.7	126	7	8.0
	-	5	8.4	114	3	3.6

[illegible]

APPENDIX A

MUTAGENESIS STUDIES OF PESTICIDE COMPOUNDS

MOUSE HERITABLE TRANSLOCATION TEST

CAPTAN

SUMMARY

SRI conducted a heritable translocation study of Captan in mice to investigate whether heritable mutagenic events occur when the compound is ingested repeatedly over an extended period.

For 8 weeks, adult male mice were administered Captan in their diet; 60 mice received 2500 ppm, and 61 received 5000 ppm. A control group of 60 adult male mice received an untreated diet during this time. A positive control group containing 66 adult male mice was treated as a control group for 4 weeks and then received the known mutagen triethylenemelamine (TEM) in the drinking water for 4 weeks. After treatment, all males were bred with two virgin females each to produce an F_1 generation, the males of which were raised to maturity. Selected (200 per group) F_1 males were bred to three virgin females each; and presumptive translocates were rebred to three additional females each. A third breeding was conducted with selected nonbreeder and/or presumptive males.

Evaluation of the data on fertility, breeding, and litter size distribution for F_0 and F_1 generations does not suggest the presence of translocation heterozygotes in control or Captan-treated male mice. Data on dead implants and rebreeding did, however, suggest the presence of translocation heterozygotes in the group treated with 5000 ppm Captan.

Meiotic cell preparations of the testes of the presumptive males were evaluated cytogenetically. Normal meiotic chromosomes were found in the following numbers of F_1 males derived from the group specified: 8 of 8 controls, 8 of 8 from the 2500 ppm Captan group, 8 from the 5000 ppm Captan group, and 2 of 2 from the 5000 ppm Captan-treated group derived from traumatized F_0 females. Five of 5 TEM-treated F_1 males and 1 of 8 from the 5000 ppm Captan-treated males showed reciprocal translocations.

The results of this study show that under the experimental procedures employed, Captan at 5000 ppm in the diet of male mice for 8 consecutive weeks can produce a heritable mutagenic event in F_1 generation male mice.

Introduction

The EPA is reviewing and evaluating the health hazard of pesticides and of substitute candidate pesticides according to available data. Additionally, the Agency is obtaining supplemental laboratory data. The objective is to enable the EPA to select those chemicals that are minimally hazardous when used according to labeling restrictions. BRI is participating in this Substitute Chemical Program by investigating the mutagenic potential of selected materials by *in vitro* and *in vivo* procedures.

Captan has been shown to respond in a positive manner in *Salmonella typhimurium*, *Escherichia coli* WP2, *Saccharomyces cerevisiae*, *E. coli* (relative toxicity), *Bacillus subtilis*, WI-38 unscheduled DNA synthesis (UDS) with metabolic activation, and *Drosophila melanogaster* experiments. It was not positive in a mouse dominant-lethal test. Based on positive responses in both Tier I (*in vitro* test) and Tier II (*Drosophila*) mutagenic studies, it was recommended that a heritable translocation test (Tier III) in the mouse be conducted to further assess the mutagenic potential of Captan.

In this study, young adult male ICR/BIN mice from a closed, random-bred colony were administered Captan in the diet for 8 weeks. After treatment, each male was mated to two virgin females to produce an F_1 generation, the males of which were raised to maturity and bred to three virgin females each. Pregnant females were evaluated against pre-determined selection criteria for identification of suspect F_1 males, which were retested and evaluated again. Presumptive F_1 males were examined cytogenetically.

Through this procedure, a heritable mutagenic response can be detected. Potential mutagenic effects were identified by examination of fetuses during the middle to later stages of gestation. Cytogenetic

examinations were made of meiotic cell preparations of the testes from suspect males for confirmation of findings obtained from the breeding studies.

Reported here are the results of the heritable translocation study of Captan.

MOUSE HERITABLE TRANSLOCATION TEST

Background

Human populations frequently are exposed to man-made chemicals, often at barely detectable levels, for extended periods. To evaluate the genetic hazards of such chemicals, a prudent approach is to study them in mammalian systems so as to maximize detection of a mutagenic response. The study reported here was such an investigation of Captan for its potential to produce heritable genetic defects.

Chemical induction of chromosomal aberrations in the mouse is a valuable and important experimental aid in understanding the many genetic defects due to chromosomal anomalies in humans. To date, mammalian evaluations of chemically induced chromosomal aberrations have been attempted with the dominant-lethal test and cytogenetic studies of somatic and germinal cells. Although these procedures can provide useful information, they do not measure heritable genetic effects, the most important mutagenic occurrences that are permanent and transmissible. A need exists for a method to reliably identify compounds that cause heritable chromosomal aberrations in mammalian systems. The mouse translocation procedure appears to be such a system.

A well-defined translocation test will demonstrate the fertility of an F_1 male population derived from F_0 males treated with a test agent. Confirmation of a nonbreeder, sterile, or partially sterile response can be obtained by cytological examination of the germ cells from suspected males. Sterility and partial sterility are closely correlated with the induction of translocation heterozygotes.

The procedure used in conducting this translocation test was based on experimental techniques described by Leonard and DeKnudt,[1] Cattanach et al.,[2] Falconer et al.,[3] and Generoso.[4] We modified this approach, in consultation with government and industry scientists actively engaged in mutagenesis research.

Materials and Methods

Animals

Male and female ICR/SIM mice were purchased from Simonsen Laboratories, Gilroy, California. The F_0 males were 8 to 10 weeks old. The females used in the breeding phases were 10- to 12-week-old virgin stock.

Chemical Supply

A supply of Captan sufficient for all aspects of the experimental program was received from Battelle Columbus Laboratory and EPA-RTP. Lot number 8X-650, Chevron Chemical Company, was used for all treatment periods. The excess material has been placed in storage in case it is needed for future reference.

Dosage Selection and Compound Administration

SRI and EPA staff selected the two dosage levels of Captan to be used in this experimental program. For 8 weeks, Captan was fed in the diet at 7500 and 15000 ppm.

An appropriate amount of Captan was dissolved and/or suspended in corn oil. Then the compound-oil concentrate was added at a level of 1% to a finely ground commercial diet (Purina) of known composition. The use of corn oil assured even distribution of Captan and prevented its stratification in an otherwise dry diet. Diets prepared at 2-week intervals were refrigerated at 5°C until fed to the animals. The diet was replaced in the feed containers twice weekly to minimize the possibility of compound loss. Body weights and food consumption were recorded weekly during the 8-week exposure period.

Reference Control

Males in the reference control group were fed the Purina diet with only corn oil added at a level of 1%. These mice were treated in the same manner as those in the compound test groups. Body weights were recorded weekly, as was food consumption.

Positive Control

For the positive control group, the known mutagen triethylenemelamine (TEM) was administered in the drinking water at 0.12 mg/liter for 2 weeks and then at 0.124 mg/liter for 2 weeks. TEM treatment was initiated after the males had been on the control diet for 6 weeks. Body weights and food consumption were recorded weekly. TEM is one of the chemical mutagens that have the demonstrated effect of inducing translocations in the F_1 progeny of F_0 treated males.

Genetic Tests

After 8 weeks of treatment, the males in each treatment group were mated to two adult virgin females each. After 1 week, each female was housed individually and allowed to deliver its litter. The F_0 males were discarded. All litters were raised to weaning age, at which time the females were discarded. The F_1 males were raised to maturity. At maturity (10 to 12 weeks of age), 200 F_1 males from each experimental group were selected randomly and housed individually.

Three adult virgin females were housed with each F_1 male for its first breeding. They were examined daily for the presence of vaginal plugs. These females were sacrificed 14 days after mating, and a uterine analysis was performed for determination of the number of total, live, and dead implants. Males bred to females that produced litters fitting our criteria for presumptive classification as sterile, partially sterile, or nonbreeder were rebred to three new virgin females each. The same evaluation was made for the second breeding.

Our criteria for presumptive classification of a male as "partially sterile," "sterile," or "nonbreeder" are:

- "Partially Sterile" Male
  - If all 3 females are pregnant, each must have 9 or fewer live implants, with at least 1 having 6 or fewer live implants.
  - If only 2 of 3 females are pregnant, both must have 9 or fewer live implants, with 1 having 6 or fewer live implants.

- If only 1 of 3 females is pregnant, this female must have 6 or fewer live implants.

- "Sterile" Male

  - None of 3 females pregnant--previously identified by presence of vaginal plug.

- "Nonbreeder" Male

  - None of 3 females pregnant--not previously identified by presence of vaginal plug.

Any F_1 male that did not fit one of these descriptions was considered "normal" and was discarded. For each F_1 male in the control and compound-treated groups suspected of being a translocate or nonbreeder after 2 or 3 breedings, a cytogenetic evaluation was made of meiotic cell preparations of its testes. Five males from the positive control group were also subjected to cytogenetic evaluation.

Evaluation of Breeding Data

F_1 males were identified as sterile, partially sterile, or nonbreeders by the methods outlined above. Individual data were totaled to give the number of observed (presumptive) translocations per treatment group, using a data base of 600 to 800 females per group. Also, for an accurate review of such findings, the F_0 breeding and litter data were thoroughly evaluated. The various measured evaluated included percentage of pregnancies, average litter size, average number of males and females, average number of males with females having zero to five or more dead implants, average number of females having zero to five or more dead implants, percentage of females with plugs, and percentage of pregnancies with and without plugs.

Meiotic Cell Cytogenetic Studies

Cytogenetic examinations were made of the testes of 11 F_1 mice, with the two testes from each mouse being examined separately. The procedures

used for the cytogenetic preparations are as follows. CO_2 was used to sacrifice the mice. The testes were removed, weighed, and placed in an isotonic solution of 2.2% sodium citrate. The tunica of each testis was punctured to release the tubules, which were then rolled on a glass plate to release the cell contents into the isotonic solution. The resulting cell suspension was centrifuged at 800 rpm for 5 minutes; the supernatant was removed, and each pellet of cells was resuspended in 5 ml of 1% sodium citrate hypotonic and held at room temperature for 15 minutes. The cells were centrifuged again at 800 rpm for 5 minutes and the supernatent was discarded. The cells were then treated with Carnoy's fixative (3 parts methyl alcohol and 1 part glacial acetic acid) to give a total volume of 5 ml, and immediately centrifuged again at 800 rpm for 5 minutes. This procedure was performed twice. Then the cells, suspended in an appropriate amount of fixative, were dropped onto clean, wet microscope slides and allowed to air-dry. The slides were stained with 2% buffered Giemsa for 5 minutes. Coverslips were attached with Permount. The slides were coded to preclude bias on the part of the scorers.

RESULTS AND DISCUSSION

General

Table 1 presents the average body weights for mice in the various groups. The body weights of the control, TEM, and 2500 ppm Captan group were within normal limits and comparable throughout the experiment. The 5000 ppm Captan-treated group showed a depressed body weight for 5 weeks before demonstrating a recovery trend during Weeks 6 to 8. This body weight depression appeared to be due to the inability of the male mice to acclimate to such a high level of compound in their diet.

Table 2 summarizes the average food consumption by treatment group. Both Captan-treated groups (2500 and 5000 ppm) showed a lower average weekly food intake than did the control and TEM-treated groups.

During the week immediately following the 1-week F_0 generation mating, one animal rack holding some females that had been mated with the mice given 5000 ppm Captan was accidentally tipped and some cages were spilled onto the floor, resulting in our inability to identify which males had been mated with these females. There were, however, sufficient members of F_1 generation males from these females that were not traumatized to allow us to randomly select 200 F_1 males for use in subsequent F_1 generation breedings. In addition, we held all females that had been traumatized by the tipping of the rack and maintained them throughout the remainder of the study as a separate group. From this separate group we selected 50 F_1 males (at least one per female retained) for use in the F_1 generation breedings and evaluations.

F_0 Generation

Information on the breeding performance, litter size, sex distribution, and clinical effects of the F_0 generation should be included in the evaluation of translocation data, because it may provide valuable reference data.

Table 4 summarizes the breeding and litter performance of the F_0 generation. No adverse effects were observed in the control and 2500 ppm Captan groups. The two 5000 ppm Captan groups showed a reduced pregnancy rate; the rate for the traumatized females was 29% below that of the controls. Litter sizes for the 5000 ppm groups were slightly below control values. As expected, the TEM group had a reduced pregnancy rate and a litter size 47% below the control level.

Table 4 presents litter-size distributions of live young from the F_0 generation mating. Although the distribution patterns for the control and Captan-treated animals were within normal ranges for our strain of mouse, there was evident a definite pattern of decreasing litter size and increasing variance and standard deviations between the different experimental groups. As expected, the TEM-treated animals showed the classic shift toward smaller litters. Figure 1 graphically presents the data on the F_0 generation litter-size distribution.

F_1 Generation

Table 5 summarizes breeding data from the first mating of the F_1 generation male mice. In the females mated with TEM males and with males from the 5000 ppm Captan group of traumatized F_0 mothers, there were 10% fewer females with mating plugs and an increased percentage of nonpregnant females in comparison with control values; also, these two male groups had an increased percentage of males with no pregnant females. Results from males in the 2500 and 5000 ppm Captan groups were within normal limits for this strain of mouse and comparable with values from control males.

Litter-size distributions of live implants derived from the first mating of F_1 generation males are presented in Table 6. Responses of control and Captan groups were within normal limits and readily comparable. The TEM group showed approximately a 13% reduction in litter size. Mean litter sizes were 11.72 for the control group, 11.71 for the 2500 ppm Captan group, 11.56 for the 5000 ppm Captan group, 11.88 for the 5000 ppm traumatized F_0 female group, and

10.4% for the TEM-treated group. The data on the F_1 generation litter-size distribution are presented graphically in Figure 2.

Tables 7 and 8 summarize the data on dead implants per F_1 male and dead implants per female, respectively. The 2500 and 5000 ppm Captan groups showed a slight increase in total dead implants for both males and females at the 4, 5, and 15 levels when compared with controls. TEM animals showed significant increases in dead implants for both males and females.

Table 9 summarizes the breeding results by treatment of those F_1 males classified as presumptive sterile, partially sterile, or non-breeders after three breedings. Table 10 identifies these F_1 males individually by number and treatment.

Details of the breeding and rebreeding data for presumptive F_1 males are presented in Table 11. In the reference control group, 24 of the 200 males were considered as presumptive translocates. When rebred, 12 males remained in this classification. A third mating of selected questionable and/or nonbreeder males reduced the number of presumptive males to eight: 1 nonbreeder, 1 presumptive sterile, and 6 partially sterile (1 of which were questionable partially sterile).

For the TEM group, 81 of 200 F_1 males were identified as presumptive mutants after the first breeding. When rebred, 53 still met the original criteria. A third breeding reduced this number to 49; 4 continued to be nonbreeders, 14 were presumptive sterile, and 31 were partially sterile (6 of which were questionable partially sterile).

In the 2500 ppm Captan group, 30 of 200 F_1 males were identified as presumptive mutants after the first breeding. When rebred, 11 still met the criteria: 5 were nonbreeders, 1 was a presumptive sterile, and 2 were partially sterile (1 of which was questionable partially sterile).

The 5000 ppm Captan group also had 30 of 200 F_1 males identified as presumptive mutants after the first breeding. The second mating reduced this number to 9. After a third breeding, 8 males still met the

original criteria: 3 were nonbreeders, 1 was a presumptive sterile, and 5 were partially sterile.

For the group of F_1 males derived from traumatized F_0 females and males treated with 5000 ppm Captan, 12 of 50 F_1 males were identified as presumptive mutants after the first mating. A second breeding reduced this number to 4 and a third breeding further reduced to 2 the number of F_1 males that still met the original criteria; both were partially sterile, with one of them being questionable partially sterile.

The data on the F_0 and F_1 generations' fertility, breeding, and litter-size distribution as well as the data on the F_1 generation's dead implants and rebreeding show that Captan tends to induce dose-related effects on the reproductive performance of male mice. The data also suggest the presence of translocation heterozygotes in the 5000 ppm Captan group.

Review of the data on dead implants, breeding, and rebreeding for the F_1 generation of the TEM-treated group showed, as expected, the potential for the presence of translocation heterozygotes in 24.5% of the F_1 males.

Cytogenetic Studies

Table 12 presents the findings from the cytogenetic evaluation of meiotic cell preparations from F_1 males in the Captan groups characterized as nonbreeder, presumptive sterile, or partially sterile. Also, eight control males and 5 of 49 TEM males were evaluated.

Whenever possible, 25 spermatocytes per testis were scored. The slides were decoded only after all scoring was completed. The results are summarized as follows:

- All eight males examined in the control group were cytogenetically normal.
- The five TEM males all showed positive reciprocal translocations.
- All eight males in the 2500 ppm Captan group were cytogenetically normal.
- Seven males in the 5000 ppm Captan group were cytogenetically normal; however, the eighth male (No. 657) showed as a positive reciprocal translocation.

- the two males examined in the 5000 ppm Captan group derived from transmitted F_0 females were cytogenetically normal.

Discussion

Increased use of the translocation procedure has revealed that a meaningful relationship exists between the incidence of dead implants in F_1 matings and the occurrence of a heritable translocation event. Previous experiments at BNL and at Oak Ridge National Laboratories have demonstrated this correlation. The following paragraphs discuss occurrences of dead implants in this study.

When females had a total implant count of less than six or when all their implants were identified as dead, we generally considered this to be a result of first breeding or of some factor other than compound treatment (such as background incidence) and excluded those females from this evaluation. Tables 13 through 17 present the total, dead, and live implantation data for suspect translocates of the control, the TCM group, and the three Captan groups.

The control group (Table 13) showed a normal implant distribution, with the exception of one F_1 male (No. 122) for whom the number of implants was high. In the TCM group (Table 14), the expected increase in dead implants and the resultant decrease in live implants occurred, although the numbers of total implants were generally normal. This pattern occurred during all breeding periods.

The 2500 ppm Captan group (Table 15) contained seven males in the first breeding with females having high dead implant counts but normal live litter size, according to the criteria. Also, 3 males showed high dead implant counts during the first breeding, with live implant counts fitting the criteria for partially sterile males. This increase in dead implant occurrence was not repeated in subsequent breeding, and all F_1 males were classified as normal after the breeding phases.

Five F_1 males in the 5000 ppm Captan group (Table 16) showed an increase in dead implants during the first breeding. In the rebreeding of these males, only male No. 657 continued to show the increase in dead implants and the resultant decrease in live implants. All other F_1 males in this group had a normal distribution of dead and live implants for all breedings.

Table 17 presents the implant data for F_1 males derived from traumatised females in the 5000 ppm Captan group. With the exception of an occasional female showing an increase in dead implants, the distribution of total, dead, and live implants was normal.

The numbers of total implantations were generally within normal limits for all experimental groups in all breedings.

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Table 1

AVERAGE BODY WEIGHTS IN GRAMS FOR MICE RECEIVING VARIOUS LEVELS OF CAPTAN IN THEIR DIETS

Week of Test	Control	TEM	Dietary Levels of Captan (ppm diet) 2500	5000
Initial	11.8	13.4	11.8	13.6
1	12.5	12.9	13.1	11.8
2	13.7	14.6	13.7	11.4
3	14.8	15.4	14.4	12.1
4	14.6	15.8	14.4	11.4
5	14.8	15.9	16.0	13.0
6	17.1	18.1	17.5	15.1
7	17.7	18.9	19.1	15.8
8	19.2	19.8	18.9	16.9

Table 3

AVERAGE FOOD CONSUMPTION FOR MICE RECEIVING
VARIOUS LEVELS OF CAPTAN IN THEIR DIETS
(Grams of Food Consumed/House/Day)

Week of Test	Control	TEM	Dietary Levels of Captan (ppm diet) 2500	5000
1	4.01	4.05	3.74	3.25
2	4.82	5.00	4.72	4.38
3	4.91	5.08	4.90	4.40
4	5.28	5.33	4.93	4.45
5	5.08	5.55	5.44	4.95
6	5.36	5.56	4.95	4.75
7	5.81	6.14	5.63	5.27
8	5.55	5.74	5.13	4.83

Table 3

TRANSLOCATION STUDY OF CAPTAN
F_0 GENERATION MICE
SUMMARY OF BREEDING AND LITTER DATA

Parameter	Control	TDM	2500 ppm	5000 ppm	5000 ppm[a]
Number of F_0 males	60	66	60	31	30
Number of F_0 females	120	132	119	61	59
Number pregnant	93	77	91	38	33
Percent pregnant	77.5	58.3	76.5	62.3	55.9
Number of nonbreeder males	8	19	8	5	—
Percent nonbreeders	13.3	28.8	13.3	16.1	—
Average live litter size	12.30	6.55	12.16	11.68	11.33
Average number of males weaned/litter	5.84	3.26	5.65	6.29	5.25

[a]Group of females accidentally tipped off rack following 1 week of mating with 5000 ppm-treated mates. These traumatized females, and their offspring, most of which could not be identified back to a particular F_0 male, were considered as a separate group for the remainder of the study.

Table 5

TRANSLOCATION STUDY OF CAPTAN
MOUSE LITTER-SIZE DISTRIBUTION OF LIVE YOUNG
DERIVED FROM F_0 GENERATION ADULTS

Litter Size	Control	TEM	Captan (ppm diet) 2500	5000	5000[a]
1	0	1	1	0	0
2	0	2	0	1	0
3	0	3	0	0	0
4	0	8	0	0	1
5	0	10	1	0	0
6	0	13	2	1	1
7	1	12	0	0	1
8	2	8	1	0	0
9	4	8	1	1	1
10	6	2	8	6	1
11	8	1	9	5	7
12	18	1	24	8	5
13	22	0	20	4	8
14	18	1	10	6	1
15	6	0	6	1	3
16	1	0	3	0	0
17	0	0	2	0	0
18	0	0	0	0	0
Mean (μ)	12.10	6.55	12.16	11.68	11.11
Variance (σ^2)	1.89	5.84	4.97	5.71	6.09
Standard Deviation (σ)	1.97	7.42	2.31	2.39	2.47

[a]Traumatized F_0 females.

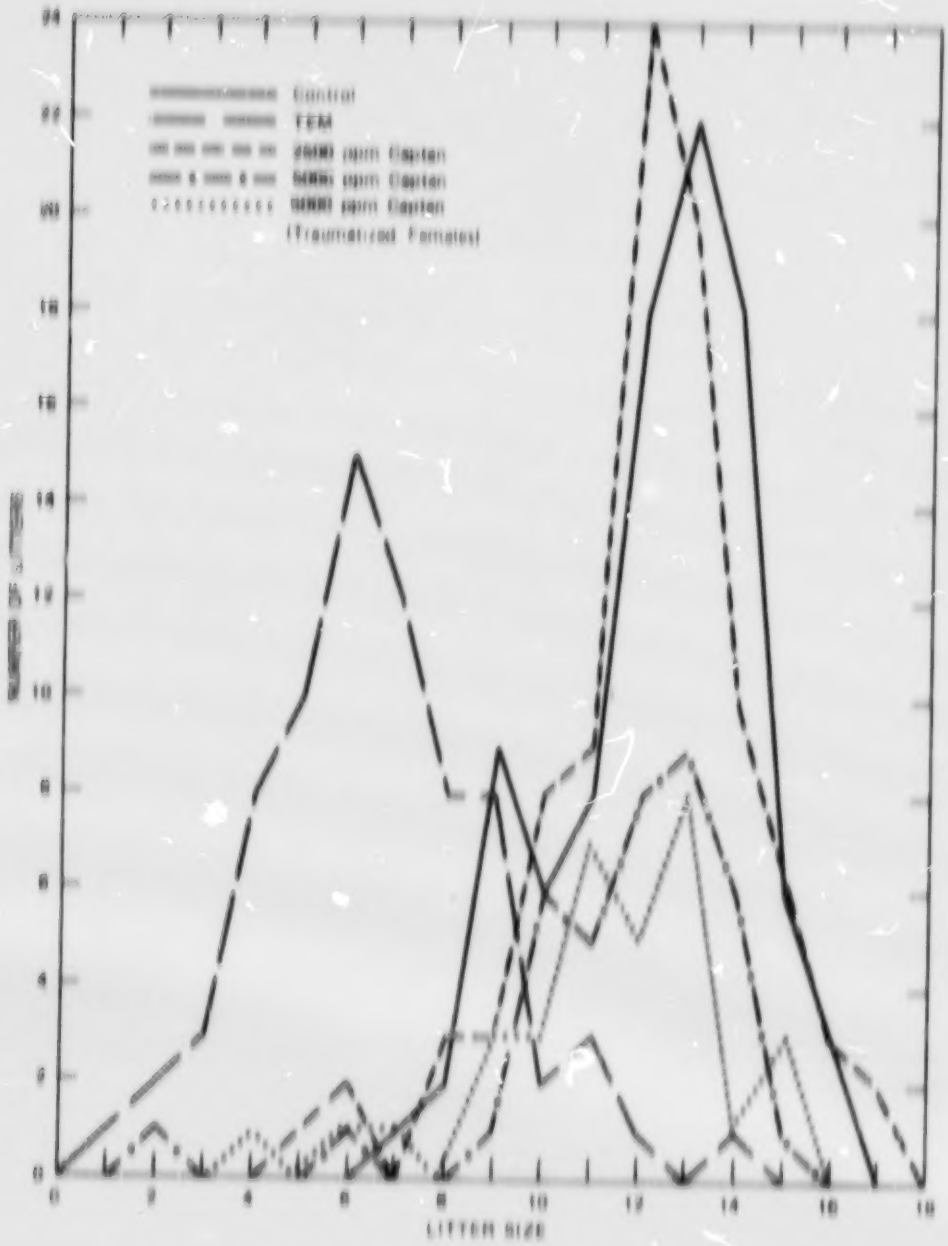

FIGURE 1 LITTER SIZE DISTRIBUTION F_0 GENERATION

Table 5

TRANSLOCATION STUDY OF CAPTAN
F_1 GENERATION MICE
SUMMARY OF BREEDING DATA--FIRST BREEDING

Parameter	Control	ZEW	Captan (ppm) 2500	5000	5000[a]
Number of F_1 males	200	200	200	200	50
Number of females	400	400	400	400	150
Number of mating plugs	430	376	442	434	65
Percent mating plugs	75	67	74	73	61
Number pregnant	402	380	421	427	112
Percent pregnant	80	76	74	86	75
Number pregnant with plug	414	326	424	417	84
Percent pregnant with plug	99	85	84	97	84
Number pregnant without plug	58	57	53	54	28
Percent pregnant without plug	12	15	11	13	26
Number not pregnant	108	217	124	147	38
Percent not pregnant	18	34	21	26	25
Number not pregnant with plug	16	50	23	24	4
Percent not pregnant with plug	15	23	19	21	3
Males with no pregnant females	13	39	14	9	7
Percent males with no pregnant females	6.5	19.5	7.0	4.5	14.0

[a]Irradiated F_0 females.

Table 6

TRANSLATION STUDY OF CAPTAN
MOUSE LITTER SIZE DISTRIBUTION OF LIVE YOUNG
DERIVED FROM F_1 GENERATION ADULTS--FIRST BREEDING

Litter Size	Control	TEM	Captan (ppm) 1500	3000	3000[a]
1	5	4	4	1	0
2	4	9	0	1	0
3	2	12	5	1	0
4	4	17	6	5	1
5	5	21	4	6	1
6	8	11	5	8	4
7	7	8	6	11	3
8	14	17	13	19	4
9	35	25	35	17	2
10	37	31	40	53	16
11	73	53	77	77	14
12	80	61	78	85	15
13	89	52	87	93	17
14	72	27	54	55	14
15	32	21	16	19	13
16	16	8	14	11	5
17	7	1	7	7	1
18	1	1	2	3	0
19	0	1	0	0	0
20	0	0	1	0	0
Mean (μ)	11.72	10.24	11.73	11.56	11.88
Variance (σ^2)	8.13	11.59	8.68	7.40	7.20
Standard Deviation (σ)	2.85	3.69	2.95	2.72	2.68

[a] Treatmented F_0 females.

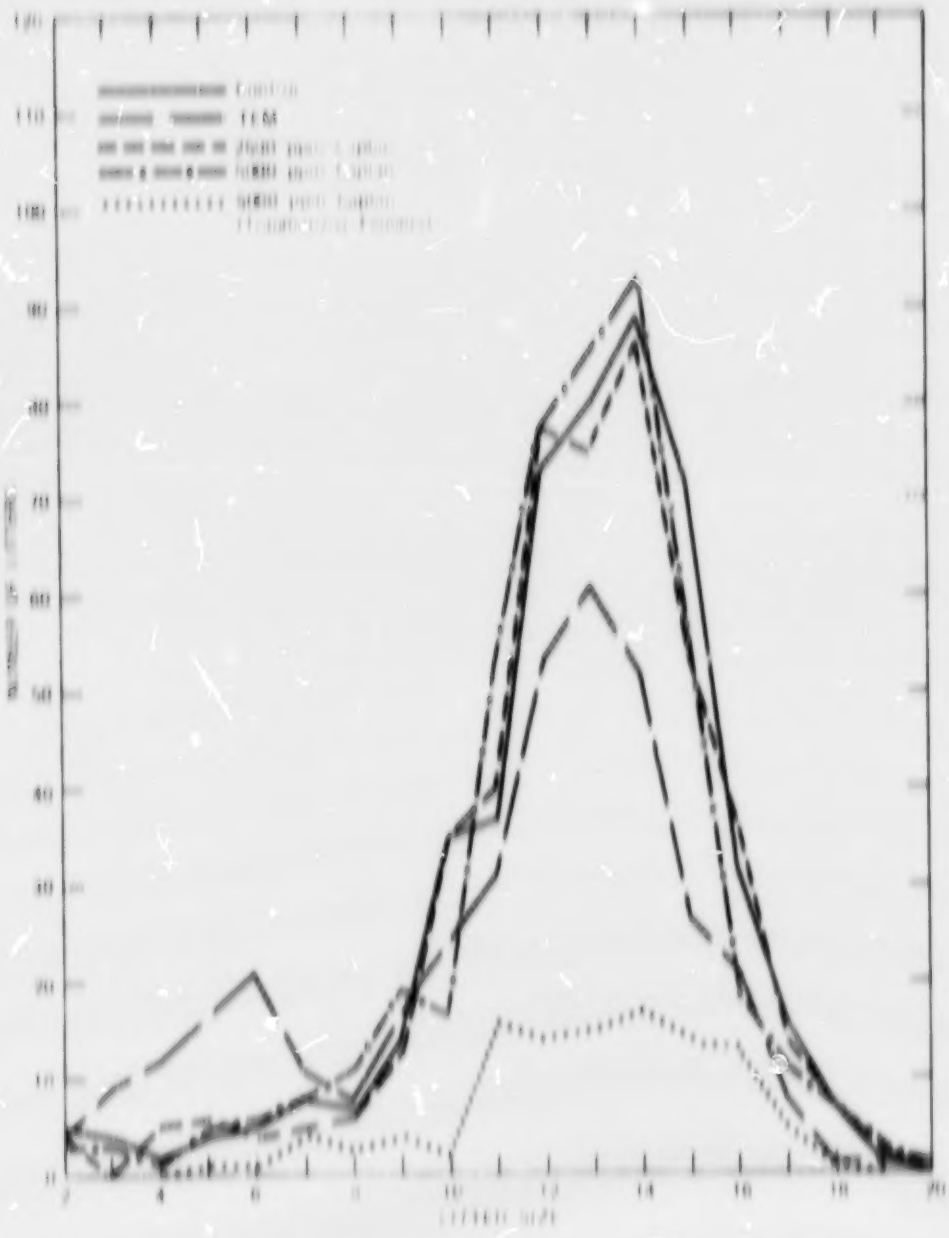

FIGURE 7 LITTER SIZE DISTRIBUTION F, GENERATION FIRST MATING

Table 7

TRANSLOCATION STUDY OF CAPTAN
SUMMARY OF DEAD IMPLANTS PER F1 MALE

Number of Males with Females Having	Control	TEM	Captan (ppm) 2500	3000	5000[a]
0 Dead Implants	68	37	69	49	20
1 Dead Implant	54	36	44	68	11
2 Dead Implants	30	25	31	36	7
3 Dead Implants	18	16	13	18	2
4 Dead Implants	8	9	10	9	0
5 Dead Implants	3	6	4	3	2
> 5 Dead Implants	7	12	7	8	1

[a]Traumatised F_0 females.

Table 8

TERATOLOGY STUDY OF CAPTAN
SUMMARY OF DEAD IMPLANTS PER FEMALE

Number of Dead Implants/Female	Control	EES	Captan (ppm) 2500	5000	5000[a]
0	320	182	295	284	79
1	109	81	111	130	23
2	19	15	17	41	9
3	18	16	16	9	0
4	1	11	7	3	0
5	0	11	2	2	0
≥ 5	1	45	2	8	1
Total Pregnant Females	492	181	473	477	112

[a]Traumatized F_0 female.

Table 9

[illegible]

	Control	[illegible]	Cancer (rad)		
			[illegible]	[illegible]	[illegible][a]
Total number of F_1 males	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Number of nonbreeder males	1	4	5	2	0
Number of presumptive sterile males	1	14	1	1	0
Number of partially sterile males	6(3?)	31(8?)	[illegible]	5	[illegible]

[a]Irradiated F_0 females

Table 10

TRANSLOCATION STUDY OF CAPTAN
INDIVIDUAL IDENTIFICATION OF PRESUMPTIVE F_1 MALES
AFTER THREE BREEDINGS

Treatment	Partially Sterile	Presumptive Sterile	Nonbreeder
Control	167	73	220
	65		
	1227		
	1647		
	189		
	190		
TEM	214	202	220
	216	231	241
	242	251	191
	2157	256	196
	235	268	
	245	271	
	262	288	
	264	317	
	269	321	
	280	326	
	281	339	
	290	341	
	292	369	
	2997	389	
	100		
	114		
	115		
	1277		
	144		
	145		
	150		
	159		
	1607		
	161		
	171		
	173		
	176		
	188		
	190		
	1997		
	4007		

(Continued)

Table 10
(Concluded)

Treatment	Partially Sterile	Presumptive Sterile	Nonbreeder
Captan 2500 ppm	680?	474	449
	526		479
			496
			521
			581
Captan 5000 ppm	635	736	634
	697		733
	760		
	779		
	780		
Captan 5000 ppm[a]	805		
	8177		

[a]Traumatised F_0 females.

Table 11

TRANSLOCATION STUDY OF CAPTAN
BREEDING AND REBREEDING SUMMARY OF PRESUMPTIVE F1 MALES
(Live Implants Only)

Treatment	F1 Male No.	First Breeding (3 Females)			Second Breeding (3 Females)			Third Breeding (3 Females)		
Control	11	-[**]	-	-	11	8	(15)[†]			
	16	-	-	(-)	-	-	-	-	-	-
	17	-	-	-	-	-	-	-	-	-
	26	-	-	-	0[*]	11	-			
	39	9	0	-	0	-	-	12	11	11
	43	-	-	-	-	-	-	10	-	-
	50	5	7	6	12	11	12			
	58	0	-	-	10	9	-			
	65	4	C	5	3	6	2			
	67	-	-	-	14	11	-			
	72	-	-	-	0	-	-	-	-	-
	86	-	-	-	(12)	-	-			
	102	-	-	(11)	9	5	16	12	12	4
	122	6	2	13	7	7	15			
	129	-	-	-	-	-	-	14	-	-
	139	-	-	-	14	-	-			
	144	-	-	-	9	10	-			
	152	1	9	9	10	8	11			
	164	-	-	-	-	-	(9)	-	-	-
	178	-	-	-	10	-	-			
	189	0	1	0	0	0	0	0	1	0
	190	1	5	6	1	0	0	0	1	1
	192	7	(14)	-	12	11	-			
	200	-	-	-	11	-	-			
	Totals		2-			12			8	
TEM	202	0	0	0	0	0	0			
	215	-	-	-	7	5	-			
	216	-	-	(9)	-	-	-	5	-	-
	220	-	-	-	-	-	-	-	-	-
	226	-	-	-	12	7	-			
	227	0	5	2	12	5	8			
	228	-	(9)	-	15	12	11			
	229	7	0	9	15	15	13			
	230	-	-	-	11	10	-			

(Continued)

[*] "0" indicates a plug was observed for a female that was not pregnant.

[**] "-" indicates a plug was not detected and the female was not pregnant.

[†] "( )" indicates all implants were in early stages of development and impossible to determine if they were live or dead upon gross observation.

Table II
(Continued)

Treatment	F1 Male No.	First Breeding (3 Females)			Second Breeding (3 Females)			Third Breeding (3 Females)		
TEM	241	(0)*	-**	-	0	0	0			
	242	1	6	1	8	4	5			
	243	-	-	-	11	13	8			
	245	8	7	6	7	7	11			
	247	5	9	8	10	10	14			
	248	1	0	1	1	0	(0)**			
	249	-	-	-	11	-	-			
	251	-	-	-	-	-	-	-	-	-
	255	-	-	-	10	13	11			
	259	(P)	0	1	1	0	-			
	261	0	0	0	0	0	0			
	263	3	10	-	15	11	-			
	264	4	-	-	11	14	11			
	266	0	-	-	-	-	-	-	-	-
	268	0	-	-	11	0	-			
	269	7	8	5	9	6	5			
	284	3	5	-	(P)	5	9			
	267	-	-	-	13	(P)	12			
	268	-	-	-	-	-	-	0	-	-
	269	-	-	-	6	7	-			
	270	0	-	-	8	-	-	9	-	-
	273	-	-	-	0	-	-	-	-	-
	280	9	7	2	8	8	5			
	281	(P)	6	-	5	1	2			
	283	9	9	8	14	0	-			
	286	-	-	-	-	-	-	10	-	-
	288	0	0	0	0	0	0			
	290	5	5	6	7	5	0			
	292	6	3	0	3	3	8			
	294	8	(18)†	9	10	15	11			
	298	8	-	-	9	6	9			
	300	6	(15)	-	8	5	2			
	302	9	-	-	13	12	15			
	314	-	-	-	2	1	4			
	315	0	1	0	0	0	0			
	317	0	0	0	0	0	-			
	318	4	4	1	9	4	11			
	321	0	0	-	0	0	-			
	322	9	-	-	11	11	-			

(Continued)

*"0" indicates a plug was observed for a female that was not pregnant.

**"-" indicates a plug was not detected and the female was not pregnant.

†"( )" indicates all implants were in early stages of development and impossible to determine if they were live or dead upon gross observation.

†† "(P)" indicates female was pregnant but had no live implants.

Table II
(Continued)

Treatment	F1 Male No.	First Breeding (3 Females)			Second Breeding (3 Females)			Third Breeding (3 Females)		
1ppm	126	-**	-	-	0*	-	-			
	127	0	-	-	0	(10)†	-	1	(9)	-
	131	0	6	-	10	0	-			
	134	-	-	-	-	-	-	12	12	-
	139	0	-	-	0	0	0			
	143	0	0	0	0	0	0			
	144	3	5	-	5	5	5			
	145	6	6	5	6	6	3			
	146	-	-	-	12	11	-			
	148	-	-	(12)	10	8	10			
	150	6	3	2	(p)††	0	1			
	155	-	-	-	11	0	-			
	156	-	-	-	11	-	(15)			
	159	0	6	8	7	3	7			
	160	(15)	-	-	2	(p)	-			
	161	6	6	9	3	7	(p)			
	163	5	-	-	12	14	-			
	167	-	-	-	6	5	11			
	169	0	-	-	-	-	-	-	-	-
	171	6	6	-	5	5	5			
	172	(16)	-	-	10	9	-			
	175	3	-	-	5	-	7			
	176	5	0	6	1	-	1			
	181	-	-	-	11	0	-			
	182	(13)	(11)	-	-	-	-	11	10	-
	185	0	5	(6)	10	12	7			
	187	-	-	-	15	-	-			
	188	3	6	0	3	3	6			
	189	-	-	-	-	-	-	0	-	-
	190	6	3	5	3	5	3			
	191	-	-	-	-	-	-	-	-	-
	196	-	-	-	-	-	-	-	-	-
	197	-	-	(15)	11	0	0			
	199	5	6	(11)	5	2	6			
	600	2	2	(12)	6	5	2			
	Totals		81			51			59	

(Continued)

*"p" indicates a plug was observed for a female that was not pregnant.

**"-" indicates a plug was not detected and the female was not pregnant.

†"( )" indicates all implants were in early stages of development and impossible to determine if they were live or dead upon gross observation.

††"p" indicates female was pregnant but had no live implants.

Table 11
(Continued)

Treatment	F1 Male No.	First Breeding (3 Females)			Second Breeding (3 Females)			Third Breeding (3 Females)		
Captan 2500 ppm	430	.[**]	.	.	12	8	12			
	432	4	(17)[†]	9	11	10	12			
	436	9	5	8	11	12	11			
	449	.	.	.	.	.	.	.	.	.
	450	0[*]	1	14	10	13	13			
	451	7	0	.	0	0	10			
	452	9	10	10	11	11	13			
	455	.	.	.	10	12	11			
	461	.	.	.	13	11	9			
	469	.	.	.	.	.	11			
	472	8	10	9	0	11	10			
	476	.	.	.	0	.	.	.	.	.
	479	.	.	.	.	.	.	.	.	.
	480	(3)	0	.	(13)	.	8	2	.	.
	484	4	14	13	11	0	12			
	489	11	12	6	9	11	14			
	496	.	.	.	.	.	.	.	.	.
	518	.	.	.	0	11	13			
	523	.	.	.	.	.	.	.	.	.
	526	7	1	(1)	0	5	0	2	1	0
	528	0	.	.	14	.	.			
	546	.	.	.	.	.	.	9	13	.
	561	.	.	.	.	.	.	15	11	
	568	8	(9)	(13)	9	11	8			
	569	.	.	.	11	.	10			
	582	.	.	.	8	13	7			
	583	.	.	.	.	.	.	.	.	.
	585	9	10	12	12	13	13			
	588	.	.	(11)	9	.	.	11	.	.
	590	8	0	9	0	11	12			
	Totals		10			11			8	
Captan 5000 ppm	601	.	(11)	(8)	11	6	.			
	604	7	9	9	9	10	14			
	620	8	.	.	9	.	.			
	622	.	.	(9)	0	12	.			
	626	.	.	.	(12)	12	.			
	627	8	8	8	10	6	(p)[††]			
	634	.	.	.	.	.	.	.	.	.
	635	.	.	.	.	(p)	.	.	.	.

(Continued)

[*] "0" indicates a plug was observed for a female that was not pregnant.

[**] "." indicates a plug was not detected and the female was not pregnant.

[†] "( )" indicates all implants were in early stages of development and impossible to determine if they were live or dead upon gross observation.

[††] "(p)" indicates female was pregnant but had no live implants.

Table 11
(Concluded)

Treatment	F1 Male No.	First Breeding (3 Females)			Second Breeding (3 Females)			Third Breeding (3 Females)		
Captan 5000 ppm	638	10	6	6	10	12	11			
	646	10	-**	-	11	13	9			
	653	0*	(13)†	-	14	13	10			
	657	2	5	1	2	1	6			
	660	0	10	0	14	9	12			
	668	-	-	6	9	12	12			
	671	-	-	-	14	11	-			
	672	-	-	-	-	-	14			
	679	6	0	8	7	12	3			
	722	-	-	-	-	-	-	12	-	-
	732	4	-	-	12	-	-			
	733	-	-	-	-	-	-	-	-	-
	735	-	-	-	11	12	-			
	736	-	-	-	-	-	-	0	-	-
	743	0	-	(15)	14	10	10			
	760	7	5	8	1	0	0			
	761	9	-	-	5	13	11			
	765	9	10	-	12	11	9			
	767	10	-	-	12	13	12			
	769	8	0	(12)	12	13	10			
	779	2	0	1	0	5	-			
	780	5	1	5	0	2	6			
	Totals		38			9			8	
Captan 5000 ppm[a]	805	-	-	-	-	-	9	-	-	-
	806	-	-	-	12	14	10			
	815	6	9	7	13	11	10			
	816	-	(10)	(11)	9	-	-			
	817	-		-	7	0	-	11	2	13
	818	10	6	-	11	8	12			
	822	-	-	-	6	-	-	10	12	12
	823	-	-	-	13	-	-			
	837	-	-	-	7	-	-	-	-	-
	841	-	-	-	13	10	-			
	846	5	-	-	10	12	12			
	847	(14)	-	-	10	10	14			
	Totals		12			6			2	

[a] Traumatized F_0 females.

* "0" indicates a plug was observed for a female that was not pregnant.

** "-" indicates a plug was not detected and the female was not pregnant.

† "( )" indicates all implants were in early stages of development and impossible to determine if they were live or dead upon gross observation.

Table 12

TRANSLOCATION STUDY OF CAPTAN
CYTOGENETIC EVALUATION OF F_1 MALE MICE

Treatment	F_1 Male No.	Body Weight (g)	Testes Weight (mg)	Classification-Based Upon Breeding Data	Cytogenetic Classification
Control	16	64.6	265	Partially sterile (questionable)	Normal
	17	58.5	307	Nonbreeder	Normal
	65	38.8	237	Partially sterile	Normal
	72	48.0	232	Presumptive sterile	Normal
	122	48.7	288	Partially sterile (questionable)	Normal
	154	55.8	314	Partially sterile (questionable)	Normal
	188	48.6	245	Partially sterile	Normal
	190	47.5	222	Partially sterile	Normal
TEM	232	54.6	276	Partially sterile	Positive reciprocal translocation
	262	42.4	243	Partially sterile	Positive reciprocal translocation
	290	62.6	222	Partially sterile	Positive reciprocal translocation
	345	54.1	284	Partially sterile	Positive reciprocal translocation
	361	58.4	278	Partially sterile	Positive reciprocal translocation
Captan	449	60.2	256	Nonbreeder	Normal
2500 ppm	474	56.5	175	Presumptive sterile	Normal
	479	65.1	287	Nonbreeder	Normal
	480	58.7	307	Partially sterile (questionable)	Normal
	496	60.9	342	Nonbreeder	Normal
	523	58.7	348	Nonbreeder	Normal
	526	54.1	295	Partially sterile	Normal
	580	58.7	297	Nonbreeder	Normal

Table 12
(Concluded)

Treatment	F_1 Male No.	Body Weight (g)	Testes Weight (mg)	Classification-Based Upon Breeding Data	Cytogenetic Classification
Captan	634	57.1	298	Nonbreeder	Normal
5000 ppm	635	56.0	312	Partially sterile	Normal
	657	62.0	231	Partially sterile	Positive reciprocal translocation
	733	62.2	236	Nonbreeder	Normal
	736	57.9	256	Presumptive sterile	Normal
	760	55.9	248	Partially sterile	Normal
	774	54.8	224	Partially sterile	Normal
	780	55.0	295	Partially sterile	Normal
Captan	805	70.0	268	Partially sterile	Normal
5000 ppm[a]	837	56.9	303	Partially sterile (questionable)	Normal

[a] Treatmented F_0 females.

Table 11

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
			Control Group			
First breeding	11	1	[a]			
		2				Nonbreeder
		3				
	16	1				
		4				Partially sterile (questionable)
		5	(1)[b]	1	(1)	
	17	1				
		2				Nonbreeder
		3				
	24	1				
		2				Nonbreeder
		3				
	39	1	9	0	9	
		2	0[c]	0	0	Normal
		3				
	43	1				
		2				Nonbreeder
		3				
	50	1	5	0	5	
		2	8	1	7	Partially sterile
		3	7	1	6	
	59	1	0	0	0	
		2				Presumptive sterile
		3				
	65	1	5	1	4	
		2	0	0	0	Partially sterile
		3	5	0	5	
	67	1				
		2				Nonbreeder
		3				
	72	1				
		2				Nonbreeder
		3				
	86	1				
		2				Nonbreeder
		3				
	102	1				
		2				Partially sterile (questionable)
		3	(1)		(1)	
	122	1	16	10	6	
		2	3	2	1	Normal
		3	11	0	11	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "--" indicates a plug was not detected and the female was not pregnant.

[c] "(1)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

Table 11 (continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
			Initial Mating			
First breeding (cont.)	129	1	[a]			
		2				Nonbreeder
		3				
	139	1				
		2				Nonbreeder
		3				
	154	1				
		2				Nonbreeder
		3				
	172	1	7	5	1	
		2	12	3	9	Partially sterile
		3	9	0	9	
	185	1				
		2				Nonbreeder
		3				
	178	1				
		2				Nonbreeder
		3				
	189	1	0[b]	0	0	
		2	1	0	1	Partially sterile
		3	0	0	0	
	190	1	1	0	1	
		2	5	0	5	Partially sterile
		3	6	0	6	
	193	1	7	0	7	
		2	(15)[c]	7	(15)	Partially sterile (questionable)
		3				
	200	1				
		2				Nonbreeder
		3				
						Final Classification
Second breeding	11	1	13	0	13	
		2	0	1	0	Normal
		3	(15)	1	(15)	
	16	1				
		2				Retest[d]
		3				
	17	1				
		2				Retest[d]
		3				

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "0,0" indicates a plug was not detected and the female was not pregnant.

[c] "(15)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

[d] See third breeding for final classification.

Table 11 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Control Group: Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	26	1	0[a]	0	0	
		2	12	1	11	Normal
		3	-[aa]	-	-	
	39	1	0	0	0	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	43	1	-	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	50	1	12	0	12	
		2	11	0	11	Normal
		3	12	0	12	
	59	1	10	0	10	
		2	12	3	9	Normal
		3	-	-	-	
	65	1	3	0	3	
		2	6	0	6	Partially sterile
		3	2	0	2	
	67	1	15	1	14	
		2	11	0	11	Normal
		3	-	-	-	
	73	1	0	0	0	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	86	1	(12)[t]	?	(12)	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	102	1	10	1	9	
		2	6	1	5	Normal
		3	16	0	16	
	122	1	14	7	7	
		2	13	7	7	Normal
		3	2	0	2	
	129	1	-	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	139	1	15	1	14	
		2	-	-	-	Normal
		3	-	-	-	
	155	1	9	0	9	
		2	10	0	10	Normal
		3	-	-	-	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[aa] "-" indicates a plug was not detected and the female was not pregnant.

[t] "(12)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

[b] See third breeding for final classification.

Table 11 (continued)

TRANSLOCATION STUDY OF LAP'AM
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	152	1	11	1	10	
		2	8	0	8	Normal
		3	15	1	13	
	164	1	-[a]	-	-	
		2	-	-	-	Rebred[b]
		3	(9)[c]	-	(9)	
	179	1	11	1	10	
		2	-	-	-	Normal
		3	-	-	-	
	189	1	0[a]	0	0	
		2	0	0	0	Rebred[b]
		3	0	0	0	
	190	1	1	0	1	
		2	0	0	0	Rebred[b]
		3	0	0	0	
	193	1	13	1	12	
		2	12	1	11	Normal
		3	-	-	-	
	200	1	11	0	11	
		2	-	-	-	Normal
		3	-	-	-	
Third breeding	16	1	-	-	-	
		2	-	-	-	Partially sterile (questionable)
		3	-	-	-	
	17	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	19	1	12	0	12	
		2	12	1	11	Normal
		3	11	0	11	
	51	1	10	0	10	
		2	-	-	-	Normal
		3	-	-	-	
	72	1	-	-	-	
		2	-	-	-	Presumptive sterile
		3	-	-	-	
	86	1	13	1	12	
		2	13	1	12	Normal
		3	9	1	8	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "-" indicates a plug was not detected and the female was not pregnant.

[c] "(9)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

[d] See third breeding for final classification.

Table 11 (concluded)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
			Control Group			
Third breeding (concl.)	179	1	15	0	15	
		2	-[a]	-	-	Normal
		3	-	-	-	
	184	1	-	-	-	Partially sterile (questionable)
		2	-	-	-	
		3	-	-	-	
	189	1	0[b]	0	0	
		2	1	0	1	Partially sterile
		3	-	-	-	
	190	1	0	0	0	
		2	1	0	1	Partially sterile
		3	1	0	1	

[a] "-" indicates a plug was observed for a female that was not pregnant.

[b] "0" indicates a plug was not detected and the female was not pregnant.

Table 15

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	TEM Group: Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
First breeding	202	1	0[a]	0	0	
		2	0	0	0	Presumptive sterile
		3	0	0	0	
	213	1	.[b]	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	216	1	.	.	.	
		2	.	.	.	Partially sterile (questionable)
		3	(9)[c]	?	(9)	
	220	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	226	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	227	1	0	0	0	
		2	11	7	5	Partially sterile
		3	11	9	2	
	228	1	.	.	.	
		2	(9)	?	(9)	Partially sterile (questionable)
		3	.	.	.	
	229	1	8	1	7	
		2	0	0	0	Normal
		3	10	1	9	
	230	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	231	1	0	0	0	
		2	.	.	.	Presumptive sterile
		3	.	.	.	
	232	1	12	9	3	
		2	9	5	7	Partially sterile
		3	10	9	1	
	233	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	235	1	13	7	8	
		2	11	5	7	Partially sterile
		3	11	5	6	
	237	1	5	0	5	
		2	12	3	9	Partially sterile
		3	12	5	8	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "." indicates a plug was not detected and the female was not pregnant.

[c] "(9)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

Table 15 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

			TEM Group			
	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
First breeding (cont.)	238	1	14	13	1	Partially sterile
		2	0[a]	0	0	
		3	9	8	1	
	239	1	-[b]	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	241	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	244	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	245	1	6	6	0	Partially sterile
		2	6	6	0	
		3	6	5	1	
	251	1	0	0	0	Presumptive sterile
		2	0	0	0	
		3	0	0	0	
	253	1	12	9	3	Normal
		2	13	3	10	
		3	-	-	-	
	254	1	6	1	5	Partially sterile
		2	-	-	-	
		3	-	-	-	
	256	1	0	0	0	Presumptive sterile
		2	-	-	-	
		4	-	-	-	
	258	1	0	0	0	Presumptive sterile
		2	-	-	-	
		3	-	-	-	
	262	1	12	10	2	Partially sterile
		2	12	6	6	
		3	17	10	5	
	264	1	8	3	5	Partially sterile
		2	8	3	5	
		3	-	-	-	
	267	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	268	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	269	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "-" indicates a plug was not detected and the female was not pregnant.

Table 14 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	1000 Group: Total Implantations	Dead Implantations	Live Implantations	Initial Classification
First breeding (cont.)	270	1	0[a]	0	0	
		2	-[b]	-	-	Presumptive sterile
		3	-	-	-	
	271	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	280	1	11	4	9	
		2	12	5	7	Partially sterile
		3	3	1	3	
	281	1	6	6	0	
		2	12	6	6	Partially sterile
		3	-	-	-	
	283	1	12	3	9	
		2	11	2	9	Normal
		3	9	1	8	
	286	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	288	1	0	0	0	
		2	0	0	0	Presumptive sterile
		3	0	0	0	
	40	1	9	4	5	
		2	15	8	5	Partially sterile
		3	11	7	4	
	292	1	15	8	6	
		2	10	7	1	Partially sterile
		3	0	0	0	
	294	1	8	0	8	Partially sterile
		2	(10)[c]	1	(10)	(questionable)
		3	11	2	9	
	299	1	11	3	8	
		2	-	-	-	Normal
		3	-	-	-	
	300	1	9	3	6	Partially sterile
		2	(13)	7	(13)	(questionable)
		3	-	-	-	
	302	1	11	3	8	
		2	-	-	-	Normal
		3	-	-	-	
	115	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "-" indicates a plug was not detected and the female was not pregnant.

[c] "()" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

Table 14 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

		TRM Group			
	Male Number[a]	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
First breeding (cont.)	3.5	1	0[b]	0	0	
		2	1	0	1	Partially sterile
		3	0	0	0	
	317	1	0	0	0	
		2	.[c]	.	.	Presumptive sterile
		3	.	.	.	
	318	1	9	5	5	
		2	11	9	5	Partially sterile
		3	15	10	5	
	321	1	0	0	0	
		2	0	0	0	Presumptive sterile
		3	.	.	.	
	322	1	10	1	9	
		2	.	.	.	Normal
		3	.	.	.	
	326	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	327	1	0	0	0	
		2	.	.	.	Presumptive sterile
		3	.	.	.	
	328	1	0	0	0	
		2	11	5	6	Partially sterile
		3	.	.	.	
	334	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	339	1	0	0	0	
		2	.	.	.	Presumptive sterile
		3	.	.	.	
	343	1	0	0	0	
		2	0	0	0	Presumptive sterile
		3	0	0	0	
	344	1	10	7	3	
		2	12	7	5	Partially sterile
		3	.	.	.	
	345	1	14	8	6	
		2	10	5	5	Partially sterile
		3	10	5	5	
	346	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	349	1	.	.	.	
		2	.	.	.	Partially sterile (questionable)
		3	(1)[d]	)	(1)	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "0" indicates a plug was not detected and the female was not pregnant.

[c] "." indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

[d] "(1)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

Table 15 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
			120 Group			
First breeding (cont.)	150	1	12	8	4	
		2	11	8	3	Partially sterile
		3	8	6	2	
	155	1	-[b]	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	156	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	159	1	15	6	9	
		2	12	8	5	Partially sterile
		3	16	8	8	
	160	1	(1)[a]	-	(1)	Partially sterile
		2	-	-	-	(questionable)
		3	-	-	-	
	161	1	12	6	6	
		2	15	10	5	Partially sterile
		3	11	2	9	
	163	1	9	4	5	
		2	-	-	-	Partially sterile
		3	-	-	-	
	167	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	169	1	0[b]	0	0	
		2	-	-	-	Presumptive sterile
		3	-	-	-	
	171	1	10	6	4	
		2	13	7	6	Partially sterile
		3	-	-	-	
	173	1	(16)	-	(16)	Partially sterile
		2	-	-	-	(questionable)
		3	-	-	-	
	175	1	16	11	1	
		2	-	-	-	Partially sterile
		3	-	-	-	
	176	1	11	6	5	
		2	0	0	0	Partially sterile
		3	10	5	6	
	181	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	

[b]'0' indicates a plug was observed but a female that was not pregnant.

[a]'(0)' indicates a plug was not detected and the female was not pregnant.

[a]'(1)' indicates all implants were in early stages of development and thus difficult to determine if they were live or dead upon gross observation.

Table 14 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
			CAP Group			
First breeding (cont.)	182	1	(1)[a]	1	(1)	Partially sterile (questionable)
		2	(1)	1	(1)	
		3	-[b]	-	-	
	185	1	9	0	9	
		2	6	1	5	Partially sterile
		3	(0)	1	(6)	
	187	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	188	1	14	11	3	
		2	9	4	5	Partially sterile
		3	0[c]	0	0	
	189	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	190	1	11	7	4	
		2	12	9	3	Partially sterile
		3	14	8	6	
	191	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	196	1	-	-	-	
		2	-	-	-	Nonbreeder
		3	-	-	-	
	197	1	-	-	-	Partially sterile (questionable)
		2	-	-	-	
		3	(16)	1	(16)	
	199	1	11	6	5	Partially sterile (questionable)
		2	9	3	6	
		3	(11)	1	(11)	
	600	1	12	10	2	Partially sterile (questionable)
		2	9	7	2	
		3	(12)	1	(12)	
						Final Classification
Second breeding	202	1	0	0	0	Presumptive sterile
		2	0	0	0	
		3	0	0	0	
	215	1	11	4	7	Partially sterile
		2	9	5	5	
		3	-	-	-	

[a] "(1)" indicates a plug was observed for a female that was not pregnant.

[b] "-" indicates a plug was not detected and the female was not pregnant.

[c] "(1)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

Table 15 (Continued)

TERATOLOGICAL STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 RATES

			150 Group			
	F_1 Rate Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	216	1	[a]			
		2				Rebred[b]
		3				
	220	1				
		2				Rebred[b]
		3				
	226	1	14	1	13	
		2	5	3	2	Normal
		3				
	227	1	12	0	12	
		2	11	7	5	Normal
		3	8	0	8	
	228	1	15	0	15	
		2	12	0	12	Normal
		3	12	1	11	
	229	1	15	1	14	
		2	15	0	15	Normal
		3	13	0	13	
	230	1	11	0	11	
		2	10	0	10	Normal
		3				
	231	1	0[c]	0	0	
		2	0	0	0	Presumptive sterile
		3	0	0	0	
	232	1	17	9	8	
		2	12	10	2	Partially sterile
		3	15	10	5	
	233	1	11	0	11	
		2	15	1	15	Normal
		3	7	1	6	
	235	1	11	4	7	
		2	13	6	7	Normal
		3	14	1	13	
	237	1	11	1	10	
		2	15	5	10	Normal
		3	14	1	13	
	238	1	9	8	1	
		2	12	10	2	Partially sterile
		3	13	13	0	
	239	1	13	0	13	
		2				Normal
		3				
	241	1				
		2				Rebred[b]
		3				

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "Rebred" indicates a plug was not detected and the female was not pregnant.

[c] See third breeding for final classification.

Table 15 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

				100 mg/kg		
	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	255	1	12	2	10	Normal
		2	13	0	13	
		3	11	0	11	
	263	1	1	0	1	Partially sterile
		2	0[a]	0	0	
		3	7	4	3	
	251	1	0	0	0	Presumptive sterile
		2	0	0	0	
		3	0	0	0	
	253	1	15	2	13	Normal
		2	11[aa]	1	11	
		3	-	-	-	
	254	1	13	0	13	Normal
		2	15	0	15	
		3	11	0	11	
	256	1	-	-	-	Retired[b]
		2	-	-	-	
		3	-	-	-	
	258	1	13	0	13	Normal
		2	0	0	0	
		3	-	-	-	
	262	1	8	3	5	Partially sterile
		2	12	9	3	
		3	10	5	5	
	264	1	9	9	0	Partially sterile
		2	11	6	5	
		3	12	3	9	
	267	1	14	1	13	Normal
		2	1	1	0	
		3	14	2	12	
	268	1	-	-	-	Retired[b]
		2	-	-	-	
		3	-	-	-	
	269	1	6	0	6	Partially sterile
		2	7	0	7	
		3	-	-	-	
	270	1	8	0	8	Retired[b]
		2	-	-	-	
		3	-	-	-	
	273	1	0	0	0	Retired[b]
		2	-	-	-	
		3	-	-	-	
	280	1	11	3	8	Partially sterile
		2	11	3	8	
		3	10	4	6	

[a]"0" indicates a plug was observed for a female that was not pregnant.

[aa]"-" indicates a plug was not detected and the female was not pregnant.

[b]Was rated breeding for final classification.

Table 14 (continued)

TERATOLOGICAL STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Test Group: Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	281	1	11	7	5	
		2	12	11	1	Partially sterile
		3	10	8	1	
	283	1	15	0	15	
		2	0[a]	0	0	Normal
		3	14[a]	0	0	
	286	1	-	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	288	1	0	0	0	
		2	0	0	0	Presumptive sterile
		3	0	0	0	
	290	1	13	6	7	
		2	8	3	5	Partially sterile
		3	0	0	0	
	292	1	9	6	3	
		2	14	11	3	Partially sterile
		3	11	3	8	
	294	1	11	1	10	
		2	12	0	12	Normal
		3	12	1	11	
	299	1	15	6	9	
		2	12	6	6	Normal
		3	12	3	9	
	300	1	13	5	8	Partially sterile
		2	5	0	5	(questionable)
		3	12	10	2	
	302	1	13	0	13	
		2	13	1	12	Normal
		3	15	0	15	
	314	1	7	5	2	
		2	12	11	1	Partially sterile
		3	13	8	5	
	315	1	0	0	0	
		2	0	0	0	Partially sterile
		3	0	0	0	
	317	1	0	0	0	
		2	0	0	0	Presumptive sterile
		3	-	-	-	
	318	1	14	5	9	
		2	9	5	4	Normal
		3	13	2	11	
	321	1	0	0	0	
		2	0	0	0	Presumptive sterile
		3	-	-	-	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "-" indicates a plug was not detected and the female was not pregnant.

[c] See third breeding for final classification.

Table 15 (continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
			TDM Group			
Second breeding (cont.)	322	1	11	0	11	
		2	11	0	11	Normal
		3	.[a]	.	.	
	326	1	0[b]	0	0	
		2	.	.	.	Presumptive sterile
		3	.	.	.	
	327	1	11	11	0	
		2	(10)[c]	7	(10)	Rebred[d]
		3	.	.	.	
	333	1	12	2	10	
		2	0	0	0	Normal
		3	.	.	.	
	334	1	.	.	.	
		2	.	.	.	Rebred[d]
		3	.	.	.	
	339	1	0	0	0	
		2	0	0	0	Presumptive sterile
		3	0	0	0	
	343	1	0	0	0	
		2	0	0	0	Presumptive sterile
		3	0	0	0	
	344	1	10	5	5	
		2	10	6	6	Partially sterile
		3	11	6	5	
	345	1	6	2	4	
		2	12	8	5	Partially sterile
		3	4	1	1	
	346	1	13	0	13	
		2	12	1	11	Normal
		3	.	.	.	
	349	1	10	.	.	
		2	11	3	8	Normal
		3	12	2	10	
	350	1	8	8	0	
		2	0	0	0	Partially sterile
		3	10	9	1	
	355	1	13	0	13	
		2	0	0	0	Normal
		3	.	.	.	
	356	1	11	0	11	
		2	.	.	.	Normal
		3	(15)	1	(15)	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "." indicates a plug was not detected and the female was not pregnant.

[c] "11" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

[d] See third breeding for final classification.

Table 15 (continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF FECUNDITIVE F_1 MALES

	F_1 Male Number	Female Number	12D Group Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	159	1	10	3	7	
		2	9	6	3	Partially sterile
		3	16	9	7	
	160	1	10	3	7	Partially sterile
		2	14	14	0	(questionable)
		3	-	-	-	
	161	1	11	10	1	
		2	14	7	7	Partially sterile
		3	8	8	0	
	163	1	13	1	12	
		2	15	0	15	Normal
		3	-	-	-	
	167	1	10	5	5	
		2	11	6	5	Normal
		3	11	0	11	
	169	1	-	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	171	1	10	5	5	
		2	11	6	5	Partially sterile
		3	14	9	5	
	173	1	11	1	10	
		2	9	0	9	Normal
		3	-	-	-	
	175	1	8	0	8	
		2	8	1	7	Partially sterile
		3	11	4	7	
	177	1	1	0	1	
		2	1	1	0	Partially sterile
		3	9	8	1	
	181	1	11	0	11	
		2	10[a]	0	10	Normal
		3	-	-	-	
	182	1	-	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	183	1	10	0	10	
		2	13	1	12	Normal
		3	-	0	7	
	187	1	15	0	15	
		2	-	-	-	Normal
		3	-	-	-	
	188	1	12	3	9	
		2	6	3	3	Partially sterile
		3	12	8	4	

[a]"0" indicates a plug was observed for a female that was not pregnant.

[b]"rb" indicates a plug was not detected and the female was not pregnant.

[b]See third breeding for final classification.

Table 15 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	ISM Group: Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	189	1	-[a]	-	-	Rebred[b]
		2	-	-	-	
		3	-	-	-	
	190	1	16	11	2	Partially sterile
		2	12	7	5	
		3	12	9	3	
	191	1	-	-	-	Rebred[b]
		2	-	-	-	
		3	-	-	-	
	196	1	-	-	-	Rebred[b]
		2	-	-	-	
		3	-	-	-	
	197	1	11	0	11	Normal
		2	0[c]	0	0	
		3	0	0	0	
	199	1	14	9	5	Partially sterile (questionable)
		2	7	5	2	
		3	4	0	4	
	400	1	9	5	4	Partially sterile (questionable)
		2	11	6	5	
		3	3	1	2	
Third breeding	216	1	9	5	4	Partially sterile (questionable)
		2	-	-	-	
		3	-	-	-	
	220	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	241	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	256	1	-	-	-	Presumptive sterile
		2	-	-	-	
		3	-	-	-	
	268	1	0	0	0	Presumptive sterile
		2	-	-	-	
		3	-	-	-	
	270	1	9	0	9	Normal
		2	-	-	-	
		3	-	-	-	
	271	1	-	-	-	Presumptive sterile
		2	-	-	-	
		3	-	-	-	

[a] "-" indicates a plug was observed for a female that was not pregnant.

[b] "0" indicates a plug was not detected and the female was not pregnant.

[c] See third breeding for final classification.

Table 13 (Concluded)

TRANSLOCATION STUDY OF CAFEMI
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Implantations	Dead Implantations	Live Implantations	Final Classification
Third breeding (cont.)	186	1	11	1	10	Normal
		2	[a]			
		3				
	127	1				Partially sterile (questionable)
		2	[b][c]		[c]	
		3				
	135	1				Normal
		2				
		3				
	169	1				Presumptive sterile
		2				
		3				
	182	1				Normal
		2				
		3				
	189	1	[a]			Presumptive sterile
		2				
		3				
	191	1				Nonbreeder
		2				
		3				
	196	1				Nonbreeder
		2				
		3				

[a] "0" indicates a plug was observed in a female that was not pregnant.

[b] "C" indicates a plug was not detected and the female was not pregnant.

[c] "[]" indicates all implants were in early stages of development and were difficult to determine if they were live or dead upon gross examination.

Table 15

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

			2500 ppm Group			
	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
First breeding	430	1	.[a]	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	432	1	0	0	0	Partially sterile
		2	(17)[b]	1	(17)	(questionable)
		3	9	9	0	
	436	1	16	5	9	
		2	7	2	5	Partially sterile
		3	11	1	9	
	449	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	450	1	0[c]	0	0	
		2	5	5	1	Normal
		3	16	0	16	
	451	1	11	4	7	
		2	0	0	0	Normal
		3	.	.	.	
	452	1	10	1	9	
		2	12	2	10	Normal
		3	11	1	10	
	455	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	461	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	469	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	472	1	9	1	8	
		2	11	1	10	Normal
		3	12	3	9	
	474	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	479	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	480	1	(3)	.	(3)	
		2	0	0	0	Partially sterile
		3	.	.	.	
	484	1	11	2	9	
		2	16	2	15	Normal
		3	11	0	11	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "(.)" indicates a plug was not detected and the female was not pregnant.

[c] "(.)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

Table 15 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

2500 ppm Group

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
First Breeding (cont.)	489	1	14	3	11	Normal
		2	13	1	12	
		3	8	2	6	
	496	1	(a)	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	518	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	521	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	526	1	7	0	7	Partially sterile
		2	5	1	3	
		3	(1)[b]	-	(1)	
	528	1	(a)	0	0	Presumptive sterile
		2	-	-	-	
		3	-	-	-	
	546	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	561	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	568	1	12	4	8	Partially sterile (questionable)
		2	(9)	-	(9)	
		3	(13)	-	(13)	
	569	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	582	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	581	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	585	1	10	1	9	Normal
		2	11	1	10	
		3	14	2	12	
	588	1	-	-	-	Partially sterile (questionable)
		2	-	-	-	
		3	(12)	-	(12)	
	590	1	11	3	8	Normal
		2	0	0	0	
		3	0	0	0	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "-" indicates a plug was not detected and the female was not pregnant.

[c] "()" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

Table 15 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
			3300 ppm Group			
Second breeding	430	1	12	0	12	
		2	8	0	8	Normal
		3	14	2	12	
	432	1	12	1	11	
		2	11	1	10	Normal
		3	13	1	12	
	436	1	12	1	11	
		2	12	0	12	Normal
		3	13	0	13	
	449	1	-[a]	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	450	1	11	1	10	
		2	13	0	13	Normal
		3	15	1	14	
	451	1	0[a]	0	0	
		2	0	0	0	Normal
		3	11	1	10	
	452	1	14	1	13	
		2	11	0	11	Normal
		3	13	0	13	
	455	1	10	0	10	
		2	12	0	12	Normal
		3	12	1	11	
	461	1	13	0	13	
		2	11	0	11	Normal
		3	11	2	9	
	469	1	-	-	-	
		2	-	-	-	Normal
		3	12	1	11	
	472	1	0	0	0	
		2	12	1	11	Normal
		3	11	1	10	
	474	1	0	0	0	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	479	1	-	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	480	1	(1)[c]	1	(1)	
		2	-	-	-	Rebred[b]
		3	0	0	0	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[a] "-" indicates a plug was not detected and the female was not pregnant.

[c] "(1)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

[b] See third breeding for final classification.

Table 15 (continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

			2500 ppm Group			
	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	484	1	11	0	11	
		2	0[a]	0	0	Normal
		3	15	2	13	
	489	1	9	0	9	
		2	13	2	11	Normal
		3	15	0	15	
	496	1	-[b]	-	-	
		2	-	-	-	Rebred[c]
		3	-	-	-	
	518	1	0	0	0	
		2	11	0	11	Normal
		3	13	0	13	
	521	1	-	-	-	
		2	-	-	-	Rebred[c]
		3	-	-	-	
	526	1	0	0	0	
		2	-	-	-	Rebred[c]
		3	0	0	0	
	528	1	15	1	14	
		2	-	-	-	Normal
		3	-	-	-	
	546	1	-	-	-	
		2	-	-	-	Rebred[c]
		3	-	-	-	
	561	1	-	-	-	
		2	-	-	-	Rebred[c]
		3	-	-	-	
	568	1	11	2	9	
		2	11	0	11	Normal
		3	8	0	8	
	569	1	11	0	11	
		2	-	-	-	Normal
		3	10	0	10	
	582	1	2	0	2	
		2	13	2	11	Normal
		3	10	1	9	
	583	1	-	-	-	
		2	-	-	-	Rebred[c]
		3	-	-	-	
	585	1	11	0	11	
		2	11	0	11	Normal
		3	11	0	11	
	588	1	3	0	3	
		2	-	-	-	Rebred[c]
		3	-	-	-	

[a]"0" indicates a plug was checked for a female that was not pregnant.

[b]"-" indicates a plug was not detected and the female was not pregnant.

[c]See third breeding for final classification.

Table 15 (Concluded)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

2500 ppm Group

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (concl.)	590	1	0[a]	0	0	
		2	11	2	11	Normal
		3	12	0	12	
Third breeding	449	1	.[b]	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	474	1	.	.	.	
		2	.	.	.	Presumptive sterile
		3	.	.	.	
	479	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	480	1	10	8	2	Partially sterile
		2	.	.	.	(questionable)
		3	.	.	.	
	496	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	523	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	526	1	2	0	2	
		2	1	0	1	Partially sterile
		3	0	0	0	
	546	1	9	0	9	
		2	13	0	13	Normal
		3	.	.	.	
	561	1	15	0	15	
		2	11	0	11	Normal
		3	.	.	.	
	583	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	588	1	12	1	11	
		2	.	.	.	Normal
		3	.	.	.	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "." indicates a plug was not detected and the female was not pregnant.

Table 16

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

5000 ppm Group

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
First breeding	601	1	-[a]	-	-	Partially sterile (questionable)
		2	(-)[b]	7	(-)	
		3	(8)	7	(8)	
	604	1	7	0	7	Normal
		2	11	2	9	
		3	14	5	9	
	620	1	8	0	8	Normal
		2	-	-	-	
		3	-	-	-	
	622	1	-	-	-	Partially sterile (questionable)
		2	-	-	-	
		3	(9)	1	(9)	
	626	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	627	1	9	1	8	Normal
		2	(-)	3	8	
		3	8	0	8	
	634	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	635	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	638	1	10	0	10	Normal
		2	6	0	6	
		3	6	0	6	
	649	1	12	2	10	Normal
		2	-	-	-	
		3	-	-	-	
	653	1	0[c]	0	0	Partially sterile (questionable)
		2	(-)	1	(-)	
		3	-	-	-	
	657	1	9	7	2	Partially sterile
		2	(-)	8	1	
		3	8	7	1	
	660	1	0	0	0	Normal
		2	(-)	1	(0)	
		3	8	0	8	
	668	1	-	-	-	Partially sterile
		2	-	-	-	
		3	7	1	6	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "-" indicates a plug was not detected and the female was not pregnant.

[c] "(-)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

Table 16 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
			5000 ppm Group			
First breeding (cont.)	671	1	.[a]	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	672	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	679	1	6	0	6	
		2	0[b]	0	0	Partially sterile
		3	12	4	8	
	732	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	742	1	5	0	5	
		2	.	.	.	Partially sterile
		3	.	.	.	
	733	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	734	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	736	1	.	.	.	
		2	.	.	.	Nonbreeder
		3	.	.	.	
	751	1	0	0	0	
		2	.	.	.	Partially sterile (questionable)
		3	(15)[c]	/	(15)	
	760	1	8	1	7	
		2	5	0	5	Partially sterile
		3	9	1	8	
	761	1	12	3	9	
		2	.	.	.	Normal
		3	.	.	.	
	765	1	12	3	9	
		2	11	1	10	Normal
		3	.	.	.	
	767	1	12	2	10	
		2	.	.	.	Normal
		3	.	.	.	
	769	1	0	0	0	
		2	.	.	.	Partially sterile (questionable)
		3	(12)	/	(12)	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "." indicates a plug was not detected and the female was not pregnant.

[c] "()" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

Table 15 (Continued)

TRANSLOCATION STUDY OF CAPTAN
INFLAMMATION SUMMARY OF PRESUMPTIVE F_1 MALES

5000 ppm Group

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
First breeding (concl.)	779	1	2	0	2	
		2	0[a]	0	0	Partially sterile
		3	1	0	1	
	780	1	5	0	5	
		2	1	0	1	Partially sterile
		3	1	0	1	
						Final Classification
Second breeding	601	1	12	1	11	
		2	0	0	0	Normal
		3	-	-	-	
	605	1	9	0	9	
		2	10	0	10	Normal
		3	15	1	14	
	620	1	10	1	9	
		2	-	-	-	Normal
		3	-	-	-	
	622	1	0	0	0	
		2	12	0	12	Normal
		3	-	-	-	
	626	1	(13)[b]	-	(13)	
		2	12	0	12	Normal
		3	-	-	-	
	627	1	13	3	10	
		2	6	0	6	Normal
		3	1	1	0	
	634	1	-	-	-	
		2	-	-	-	Rebred[c]
		3	-	-	-	
	635	1	-	-	-	
		2	1	1	0	Rebred[c]
		3	-	-	-	
	638	1	10	0	10	
		2	12	0	12	Normal
		3	11	0	11	
	656	1	11	0	11	
		2	13	0	13	Normal
		3	10	1	9	
	657	1	15	0	15	
		2	13	0	13	Normal
		3	10	0	10	

[a] "0" indicates a plug was detected for a female that was not pregnant.

[b] "0" indicates a plug was not detected and the female was not pregnant.

[b] "(13)" indicates all implants are in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

[c] Not tested breeding for final classification.

Table 16 (Continued)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

			5000 ppm Group			
	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	657	1	7	5	2	
		2	9	8	1	Partially sterile
		3	5	1	4	
	660	1	15	0	15	
		2	10	1	9	Normal
		3	12	0	12	
	668	1	9	0	9	
		2	13	1	12	Normal
		3	13	1	12	
	671	1	15	0	15	
		2	11	0	11	Normal
		3	-[a]	-	-	
	672	1	-	-	-	
		2	-	-	-	Normal
		3	15	0	15	
	679	1	7	0	7	
		2	12	0	12	Normal
		3	5	1	4	
	722	1	-	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	732	1	12	0	12	
		2	-	-	-	Normal
		3	-	-	-	
	733	1	-	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	736	1	11	0	11	
		2	12	0	12	Normal
		3	-	-	-	
	746	1	-	-	-	
		2	-	-	-	Rebred[b]
		3	-	-	-	
	753	1	15	0	15	
		2	10	0	10	Normal
		3	11	1	10	
	760	1	1	0	1	
		2	0[c]	0	0	Partially sterile
		3	0	0	0	
	761	1	5	0	5	
		2	14	1	13	Normal
		3	12	1	11	
	765	1	15	3	12	
		2	11	0	11	Normal
		3	9	0	9	

[a]"-" indicates a plug was observed for a female that was not pregnant.

[c]"0" indicates a plug was not detected and the female was not pregnant.

[b]See third breeding for final classification.

Table 16 (Concluded)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

		5000 ppm Group			
	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
Second breeding (cont.)	767	1	15	3	12	Normal
		2	11	0	11	
		3	12	0	12	
	769	1	12	0	12	Normal
		2	11	0	11	
		3	10	0	10	
	779	1	0[a]	0	0	Partially sterile
		2	5	0	5	
		3	-[b]	-	-	
	780	1	0	0	0	Partially sterile
		2	7	0	7	
		3	5	1	4	
Third breeding	634	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	645	1	-	-	-	Partially sterile
		2	-	-	-	
		3	-	-	-	
	722	1	11	1	11	Normal
		2	-	-	-	
		3	-	-	-	
	733	1	-	-	-	Nonbreeder
		2	-	-	-	
		3	-	-	-	
	736	1	0	0	0	Presumptive sterile
		2	-	-	-	
		3	-	-	-	

[a] "0" indicates a plug was observed for a female that was not pregnant.

[b] "-" indicates a plug was not detected and the female was not presumed

Table 17

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Initial Classification
			3000[a] ppm Group			
First breeding	805	1	-[b]	-	-	
		2	-	-	-	Nonbreeder
		4	-	-	-	
	806	1	-	-	-	
		2	-	-	-	Nonbreeder
		4	-	-	-	
	815	1	5	0	5	
		2	9	0	9	Partially sterile
		4	7	0	7	
	816	1	-	-	-	
		2	(10)[c]	-	(10)	Partially sterile
		4	(11)	-	(11)	(questionable)
	817	1	-	-	-	
		2	-	-	-	Nonbreeder
		4	-	-	-	
	818	1	10	0	10	
		2	6	0	6	Normal
		4	-	-	-	
	822	1	-	-	-	
		2	-	-	-	Nonbreeder
		4	-	-	-	
	823	1	-	-	-	
		2	-	-	-	Nonbreeder
		4	-	-	-	
	837	1	-	-	-	
		2	-	-	-	Nonbreeder
		4	-	-	-	
	851	1	-	-	-	
		2	-	-	-	Nonbreeder
		4	-	-	-	
	856	1	5	0	5	
		2	-	-	-	Partially sterile
		4	-	-	-	
	857	1	(15)	7	(15)	
		2	-	-	-	Partially sterile (questionable)
		4	-	-	-	
						Final Classification
Second breeding	805	1	-	-	-	
		2	3	0	3	Nonbred[d]
		4	-	-	-	

[a] Indicates terminated F_0 females.

[b] "-" indicates a plug was observed for a female that was not pregnant.

[c] "( )" indicates a plug was not detected and the female was not pregnant.

[d] "(11)" indicates all implants were in early stages of development, and thus difficult to determine if they were live or dead upon gross observation.

[e] See first breeding for final classification.

(Table 17 continued)

TRANSLOCATION STUDY OF CAUCAO
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

	F_1 Male Number	Female Number	10d[1] Implantations	10d[2] Implantations	11d Implantations	Final Classification
Second breeding trimester	806	1	11	1	12	
		2	15	0	15	Normal
		3	10	0	10	
	815	1	13	1	14	
		2	11	0	11	Normal
		3	13	1	10	
	816	1	11	1	4	
		2	23	1		Normal
		3	1	1		
	817	1	11	1	1	
		2	0[3]	0	0	Rebred[4]
		3	1	1		
	818	1	11	0	11	
		2	8	0	8	Normal
		3	13	1	13	
	823	1	6	1	6	
		2	1	1		Rebred[4]
		3	1	1		
	824	1	13	0	13	
		2	1	1		Normal
		3	1	1		
	827	1	9	1	9	
		2	1	1		Rebred[4]
		3	1	1		
	841	1	15	1	15	
		2	10	0	10	Normal
		3	1	1		
	846	1	10	0	10	
		2	13	0	13	Normal
		3	12	0	12	
	847	1	11	1	10	
		2	10	0	10	Normal
		3	13	0	13	
Third breeding	805	1	1	1	1	
		2	1	1		Partially sterile
		3	1	1		
	813	1	11	1	11	
		2	8	1	8	Normal
		3	11	0	11	
	823	1	10	0	10	
		2	11	0	11	Normal
		3	12	0	12	

[1]Indicates translocated F_0 females.

[2]"0" indicates a plug was observed but a fetus was not present.

[3]"0" indicates a plug was not detected and the female was not pregnant.

[4]See third breeding for final classification.

Table 17 (Concluded)

TRANSLOCATION STUDY OF CAPTAN
IMPLANTATION SUMMARY OF PRESUMPTIVE F_1 MALES

			3000[a] ppm Group			
	F_1 Male Number	Female Number	Total Implantations	Dead Implantations	Live Implantations	Final Classification
Third breeding (cont.)	817	1	,,[b]			Normal
		2	"	"	"	
		3	"	"	"	

[a] Indicates traumatized F_0 females.

[b] " indicates a plug was not detected and the female was not pregnant.